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Determining the effects of some plant leaves and harvest residues on feed value and methane production in ruminant

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Abstract

This study investigated the potential use of hazelnut, black cherry, fig tree leaves, tomato, pepper, and eggplant harvest residues as feed resources or feed additives. Molasses and ecomass were added to vegetable harvest residues. The nutrient composition, *in vitro* gas production, and methane production of the feeds were determined. The *in vitro* gas production technique (Hohenheim gas test) was used to determine the gas production of the feeds. The experiment was conducted according to a completely randomized design. Among vegetable residues, eggplant had the highest crude protein (CP) content, while additives increased CP and reduced condensed tannin levels in all residues ($P<0.05$). Regarding neutral detergent fiber (NDF), additives decreased values in eggplant and pepper residues, potentially enhancing the feed intake of the animals. Fig leaves demonstrated the highest CP and lowest lignin contents among tree leaves, with superior *in vitro* true digestibility (IVTD) and energy values. Hazelnut and black cherry leaves exhibited high tannin levels, limiting their suitability as roughage, but their potential to reduce methane production was noted. Methane production was lowest in tomato residues, while fig leaves demonstrated the highest energy and IVTD values ($P<0.05$). As a result, hazelnut leaves and tomato residues have a significant effect on reducing methane production. The findings suggest that vegetable residues, particularly eggplant and pepper and tree leaves like fig, can serve as secondary roughage sources in ruminant diets, offering sustainable alternatives for reducing methane emissions. Future studies should explore different additives and their effects on nutritional and environmental parameters.

Keywords: Tomato; Pepper; Eggplant; Waste; Leaves; Methane

1. Introduction

Efficient utilization of roughage resources is critical for enhancing profitability in animal production, particularly as the reliance on expensive concentrated feeds poses economic challenges. When high-quality roughage is unavailable, various industrial by-products, pulps, and the stems and straws of certain grains or legumes are often employed as alternative feed sources. In this context, it is thought that the harvested stems and leaves of commonly cultivated vegetables such as tomatoes, peppers, and eggplants, which remain as field harvest residue and cannot be added to the economy, and the leaves of fig, hazelnut, and cherry laurel trees, which are known to be consumed by animals in the north of Turkiye (Black Sea Region), are potential roughage sources in the nutrition of ruminants.

It is known that if the roughage used in ruminant feeding is of low quality, enteric methane production is higher than that of high-quality roughage sources and causes significant environmental problems regarding global warming. In addition, methane production results in wasted feed energy and less energy utilization in animal production [1,2]. Therefore, determining the feed value, nutrient content, and methane emissions of roughage sources is of environmental importance.

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In this study, the usability of hazelnut, black cherry, and fig tree leaves, tomato, pepper, and eggplant field harvest wastes, which have an important forage potential in the Black Sea Region, as an alternative forage source in animal feeding or as a feed additive was investigated. In addition, it aimed to increase the nutritional value, methane production, and digestibility by adding some additives (molasses and ecomass) to vegetable harvest residues. The study hypothesizes that the additives will reduce the methane production of alternative forage sources and improve their feed value and digestibility.

2. Material and methods

Hazelnut, black cherry and fig tree leaves, tomato, pepper and eggplant field harvest residues used in this study were provided from Samsun and Giresun provinces (3 different places). No additives were used in tree leaves, whereas molasses and ecomass were used to increase the nutritional value of vegetable residues. After the samples were dried, they were ground, and nutrient analyses were performed. The *in vitro* gas production technique (Hohenheim gas test) was used to determine the methane production of feeds. Rumen fluid (pH=6.08-6.20) used in the experiment was taken from a bull (two-year-old Charolais crossbred, 650-700 kg live weight) recently slaughtered in a slaughterhouse operating in Samsun province.

2.1. Establishment of treatment groups

In this study, nine groups (3×3) will be formed for three different vegetable residues by adding molasses (5%) and ecomass (5%), along with a control group for each (Table 1). Additionally, three groups will be created for three different tree leaves, resulting in 12 groups in the study.

Table 1 Treatment groups in the experiment

Experimental Groups	Treatments
Tomato harvest residues	Control
	Molasses (5%)
	Ecomass (5%)
Pepper harvest residues	Control
	Molasses (5%)
	Ecomass (5%)
Eggplant harvest residues	Control
	Molasses (5%)
	Ecomass (5%)

2.2. Determination of nutrient content

The feed samples were ground to pass through a 1 mm sieve, after which dry matter (DM), crude protein (CP), and ash analyses were performed according to the methods described by AOAC [3]. Ether extract (EE) was analyzed using the ANKOM^{XT15} extraction system (Ankom Technology Corp.), following the procedure outlined by Kutlu [4]. Acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), and crude fiber (CF) were determined using the ANKOM^{2000} Fiber Analyzer (Ankom Technology, Macedon NY) in accordance with the Ankom Technology method [5]. Organic matter (OM), nitrogen-free extract (NFE), cellulose (CEL), and hemicellulose (HCEL) values were calculated. The condensed tannin (CT) content of feed samples was analyzed according to [6].

2.3. Determination of forage quality

The relative feed value (RFV) was used to determine the roughage quality of forage plants. In terms of RFV, "5" indicates poor quality to be rejected, "1" indicates good quality, and "prime" indicates the best quality [7].

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

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

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

2.4. Determination of *in vitro* gas production

In vitro gas production technique (Hohenheim gas test) was modified and applied as follows to determine the total gas production of the feeds [8, 9, 10]. Accordingly, after the feed sample was ground to pass through a 1 mm sieve, approximately 250 mg of air-dry feed material (200 mg DM) was weighed and placed in the 100 ml glass syringe. Incubations were started in the morning, and readings were taken at 3, 6, 9, 12, 24, 48, 72, and 96 hours as quickly as possible to avoid temperature changes. When the total gas production exceeded 90 ml, the accumulated gas was expelled, and this value was recorded and taken into account in the calculations. The changes in activity and composition in rumen fluid were controlled with three parallel measurements. The changes in activity and composition in rumen fluid were controlled by measuring rumen fluid, hay standard, and medium incubation without feed (blank). The gas production (GP) amounts were determined according to the following formula:

$$GP\ (ml/200mg\ DM,\ 24\ h) = [(V_{24} - V_0 - GP_0) \times 200 \times (Fk + Fc) / 2] / SW$$

Where; V_0: position of the piston at the beginning of incubation, ml; V_{24}: position of the piston after 24 hours of incubation, ml; GP_0: Average gas production of unsampled rumen fluid after 24 hours of incubation, ml; SW: weight of the tested sample in mg DM.

Gas production parameters were calculated using the PC package program NEWAY according to the model reported by Ørskov and McDonald [11].

$$y = a+b(1- e^{-ct})$$

Where a: amount of gas consisting of the immediately soluble fraction (ml), b: the amount of gas formed depending on time (ml), c: gas production rate, (ml/h), a+b: total gas production (ml), t: incubation period (hours) and y: "t" represents the gas production at the time.

Organic matter digestibility (OMD, %) was calculated from the amount of gas production (GP), crude protein (CP, DM%), and ash (DM%) at 24 hours using the formula [8]:

$$OMD,\ Roughages\ \% = 14.88 + 0.8893\ GP + 0.448\ CP + 0.651 Ash$$

The net energy for lactation (NE_L) content of the feeds, for which the gas production was determined using the gas production technique, was calculated using the equation provided below [10].

$$NE_L,\ Roughages\ (MJ/kg\ DM) = 0.101GP + 0.051CP + 0.112EE$$

GP: the volume of gas produced per 200 mg of dry matter of the feed during a 24-hour incubation period.

The metabolizable energy (ME) content was calculated based on the CP (DM%), EE (DM%), and ash (DM%) content of the feed samples using the equation provided below:

$$ME,\ (MJ/kg\ DM) = 2.20+0.136GP + 0.0574CP+0.002859\ EE^2$$

The standard for the rumen fluid collected from slaughtered animals was determined by measuring the pH of the rumen fluid using a digital pH meter (HANNA INSTRUMENTS 1332 model pH meter) without delay, with the temperature remaining constant and taking three repetitions for each measurement.

2.5. Determination of methane production

The methane production of the feeds used in the experiment was measured using an infrared methane analyzer (Sensor Europa GmbH, Erkrath, Germany model) as described by Goel et al. [12]. After reading the total gas production obtained from 24 hours of fermentation using the *in vitro* gas production technique, the gas accumulated in the syringes was transferred to the methane analyzer via a special tube for measurement. Methane production (ml) was determined as a percentage of the total gas, and methane production was calculated as follows:

Methane production (ml) = Total gas production (ml) × Methane percentage (%).

2.6. Determination of *in vitro* true digestibility

In vitro digestibility measurements were carried out using rumen fluid obtained from the same animal used in the *in vitro* gas production technique. In addition to rumen fluid, two handfuls of solid rumen contents were added and transported to the laboratory in thermos containers at 39 °C. The contents were thoroughly mixed, strained, and then used. In the Daisy incubator, each Ankom F57 bag, which does not contain nitrogen, was filled with 1 mm sieved feed samples, and all feeds were tested in triplicates. The feed samples were incubated in the incubator with CO_2 tubes, and the samples were incubated for 48 hours for the experiment, after which the results were evaluated. The nutrient content analyses of the feeds and residues were determined according to the methods outlined by AOAC [3], and the *in vitro* true digestibility (IVTD) of the feeds were assessed using the filter bag technique [13] in the Daisy incubator [14].

In vitro true digestibility (IVTD, %) = 100 – ((W3 – (W1 × C1)) × 100) / W2

Where : W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis, C1: The bag without sample was also prepared for correction.

2.7. Statistical Analysis

The data obtained from the study were evaluated using the SPSS 17.0 package program. In the experiment, a complete randomized design was applied to compare the differences between the treatment groups. Duncan's multiple range test was used for the comparison of mean values ($P<0.05$) [15].

3. Results and discussion

The nutrient contents of the vegetable residues used in the experiment are given in Table 2. The nutrient contents of tree leaves are given in Table 3. Among the vegetable residues, eggplant showed the highest values in terms of CP content (19.13% DM), molasses and ecomass added as additives in the study increased CP contents in all vegetable residues. In terms of NDF content, which is an indicator of feed intake, the use of additives in tomato residues did not affect feed intake but decreased the NDF value in eggplant and pepper residues, meaning that it can be said that feed intake will increase.

Among tree leaves, the highest CP content was observed in fig leaves (15.83% DM), but hazelnut tree leaves (13.74% DM) and cherry laurel tree leaves (13.68% DM) showed statistically lower values than fig leaves. However, it was determined that these leaves have high CP contents. Tree leaves were found to be similar to each other in terms of NDF contents, but the differences between them were not found to be significant. ($P>0.05$). In terms of lignin contents, fig leaves were again the leaves showing the lowest value, while hazelnut leaves showed the highest value in ADL contents compared to other leaves.

Table 2 Effect of additives on nutrient content and cell wall structural elements of some vegetable harvest residues, DM%

Treatments	DM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL	HCEL	CEL
TSL	91.58±0.04a	12.38±0.27cde	7.09±0.48f	0.33±0.07c	40.28±0.23c	39.92±0.80ab	62.88±0.61a	47.32±0.41a	7.48±0.58d	15.56±0.72a	39.85±0.97a
TSLM	85.84±0.01g	12.28±0.06de	9.04±0.14e	0.54±0.13c	42.80±0.18b	35.34±0.14de	61.35±1.53a	47.99±1.36a	10.2±0.29bc	13.36±0.21ab	37.79±1.11a
TSLE	91.32±0.04b	11.46±0.20f	9.40±0.45e	0.30±0.07c	44.76±0.23a	34.08±0.56e	62.24±1.23a	47.79±0.91a	10.11±0.28bc	14.45±0.49ab	37.68±0.64a
ESL	87.52±0.09f	11.97±0.17e	19.13±0.25b	1.48±0.16a	38.41±0.77d	29.01±0.95f	53.35±1.31b	40.23±0.70b	11.13±0.27b	13.13±0.89b	29.09±0.44b
ESLM	82.86±0.04i	12.44±0.05cd	20.57±0.30a	0.81±0.20bc	30.68±0.47g	35.50±0.02de	46.01±0.59c	36.31±0.68c	10.65±0.96bc	9.70±0.12c	25.66±1.12cd
ESLE	88.05±0.03e	12.8±0.02bc	20.89±0.15a	0.60±0.12c	28.81±0.38h	36.91±0.58cd	45.71±0.38c	35.74±0.13c	9.05±0.13cd	9.97±0.27c	26.69±0.24c
PSL	88.37±0.04d	12.75±0.05bc	11.21±0.09d	1.36±0.32a	34.08±0.19ef	40.59±0.39a	53.32±2.37b	40.69±0.93b	13.21±1.22a	12.64±1.65b	27.47±0.33bc
PSLM	83.23±0.05h	13.12±0.09ab	12.95±0.16c	0.37±0.15c	35.37±0.81e	38.20±0.90bc	45.02±0.33c	35.54±0.54c	11.92±0.27ab	9.47±0.81c	23.63±0.80d
PSLE	88.92±0.03c	13.34±0.07a	12.45±0.30c	1.25±0.18ab	33.31±0.57f	39.66±0.06ab	46.30±0.66c	36.04±0.42c	12.09±0.62ab	10.26±0.28c	23.95±0.34d
SL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

P<0.05; a,b…, differences between means with different letters in the same column are significant. TSL: tomato stems and leaves, TSLM: tomato stems and leaves with molasses addition, TSLE: tomato stems and leaves with ecomass addition, ESL: eggplant stems and leaves, ESLM: eggplant stems and leaves with molasses addition, ESLE: eggplant stems and leaves with ecomass addition, PSL: pepper stems and leaves, PSLM: eggplant stems and leaves with molasses addition, PSLE eggplant stems and leaves with ecomass addition, DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: Nitrogen free-extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, HCEL: hemicellulose, CEL: cellülose, SL: significant level.

Table 3 Nutrient contents and cell wall structural elements of some tree leaves, DM%

Leaves	DM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL	HCEL	CEL
FTL	88.48±0.09c	15.23±0.02a	15.83±0.14a	3.67±0.45a	26.89±0.25a	38.38±0.62c	47.07±1.80	24.48±0.23b	8.84±0.80b	22.59±1.94	15.64±0.58ab
HTL	89.14±0.07b	8.61±0.00b	13.74±0.04b	3.72±0.3a	24.24±0.32b	49.69±0.34b	49.89±1.03	31.45±0.58a	17.55±0.41a	18.44±0.68	13.91±0.28b
CTL	89.45±0.05a	6.77±0.01c	13.68±0.05b	1.24±0.58b	19.24±0.24c	59.07±0.67a	44.65±1.77	26.54±1.39b	9.33±0.44b	18.12±0.93	17.21±1.08a
SL	0.000	0.000	0.000	0.013	0.000	0.000	0.140	0.004	0.000	0.091	0.049

P<0.05; a,b…, differences between means with different letters in the same column are significant. FTL: fig tree leaves, HTL: hazelnut tree leaves, CTL: cherry tree leaves, DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: Nitrogen free-extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, HCEL: hemicellulose, CEL: cellülose, SL: significant level.

The DMD, DMI, and RFV values, roughage quality classes, condensed tannin contents, and *in vitro* true digestibility of some vegetable harvest residues and some tree leaves examined in the study are given in Table 4 and Table 5. The addition of additives to tomato residue did not affect the roughage quality of vegetable harvest residues, but the additives increased the RFV index in eggplant and pepper residues. The same situation was valid for both DMI and DMD values for vegetable residues. In terms of *in vitro* true digestibility (IVTD), no significant effect of additive supplementation was observed in eggplant and pepper vegetable residues. However, the addition of ecomass to tomato residues improved the IVTD value compared to molasses supplementation, though this improvement was statistically insignificant when compared to the control group. No difference was observed in DMI among the tree leaves. However, black cherry leaves exhibited higher RFV than hazelnut leaves. No significant difference in RFV was found between fig leaves and the other leaves. Regarding IVTD, fig leaves demonstrated the highest value, while hazelnut leaves showed the lowest. Significant differences were found among all leaves regarding IVTD (P<0.05).

Table 4 Effect of additives on forage quality and *in vitro* true digestibility of some vegetable harvest residues, DM%

Treatments	DMD (%)	DMI (% LW)	RFV	Forage Quality Classes	Condensed Tannin DM%	IVTD, DM%
TSL	52.04±0.32c	1.91±0.02c	76.99±0.88c	4	0.26±0.01d	43.53±0.20bc
TSLM	51.52±1.06c	1.96±0.05c	78.29±3.55c	4	0.44±0.02c	41.15±1.79c
TSLE	51.67±0.71c	1.93±0.04c	77.33±2.50c	4	0.28±0.07d	46.69±1.23b
ESL	57.56±0.55b	2.25±0.05b	100.52±3.20b	3	0.58±0.02ab	56.5±0.36a
ESLM	60.61±0.53a	2.61±0.03a	122.62±2.60a	1 (good)	0.56±0.06bc	55.1±3.11a
ESLE	61.06±0.11a	2.63±0.02a	124.28±1.22a	1 (good)	0.42±0.00c	58.85±0.22a
PSL	57.21±0.72b	2.26±0.10b	100.29±5.58b	3	0.70±0.04a	57.06±1.37a
PSLM	61.21±0.42a	2.67±0.02a	126.49±0.63a	Prime	0.56±0.07bc	58.55±0.96a
PSLE	60.82±0.33a	2.59±0.04a	122.26±2.35a	1 (good)	0.51±0.04bc	58.29±0.22a
SL	0.000	0.000	0.000		0.000	0.000

P<0.05; a,b…, differences between means with different letters in the same column are significant. TSL: tomato stems and leaves, TSLM: tomato stems and leaves with molasses addition, TSLE: tomato stems and leaves with ecomass addition, ESL: eggplant stems and leaves, ESLM: eggplant stems and leaves with molasses addition, ESLE: eggplant stems and leaves with ecomass addition, PSL: pepper stems and leaves, PSLM: eggplant stems and leaves with molasses addition, PSLE eggplant stems and leaves with ecomass addition, DMD: dry matter digestibility, DMI: dry matter intake, LW: live weight, RFV: relative feed value, IVTD: *in vitro* true digestibility, SL: significant level. In determining forage quality, an RFV score of "5" indicates very poor quality that is unsuitable for use, while "1" represents good quality, and "prime" denotes the highest quality standard [7].

Tannin content is an important factor influencing the consumption of roughage. In this study, among the vegetable residues, the highest condensed tannin contents were observed in eggplant and pepper residues without additives, while tomato residues without additives had lower tannin content. Overall, it was determined that the addition of additives reduced the condensed tannin content. Furthermore, the condensed tannin levels identified in the vegetable residues were generally low, indicating that they would not adversely affect feed intake in ruminants. Among the tree leaves, fig leaves exhibited the lowest condensed tannin content (3.94% DM). In contrast, the tannin levels determined for hazelnut leaves (18.72% DM) and black cherry leaves (18.35% DM) were at levels that would not be acceptable for ruminants. A study by Yuksel et al. [16] also reported the potential use of fig leaves for herbal tea, suggesting that fig leaves, unlike other tree leaves, can serve as a roughage source. However, hazelnut and black cherry leaves cannot be used as a source of roughage; they may be utilized in limited quantities to reduce methane production. Indeed, Unver et al. [7] reported that tannin levels of 1–4% in cattle rations, 6% in sheep rations, and 8–10% in goat rations are tolerable for animals. Considering this, the condensed tannin content exceeding 10% in hazelnut and black cherry tree leaves might make them less palatable for animals. Nevertheless, when used in appropriate doses, these leaves could potentially serve as an effective means to reduce methane production.

Table 5 Forage quality and *in vitro* true digestibility of some tree leaves

Leaves	DMD (%)	DMI (% LW)	RFV	Forage Quality Classes	Condensed Tannin DM%	IVTD. DM%
FTL	69.83±0.18a	2.56±0.10	138.4±5.06ab	Prime	3.94±0.18b	80.49±0.27a
HTL	64.4±0.45b	2.41±0.05	120.19±3.21b	1 (good)	18.72±0.20a	69.75±1.15c
CTL	68.23±1.08a	2.70±0.11	142.73±7.54a	Prime	18.35±0.82a	75.45±0.06b
SL	0.004	0.147	0.061	-	0.000	0.000

P<0.05; a,b…, differences between means with different letters in the same column are significant. FTL: fig tree leaves, HTL: hazelnut tree leaves, CTL: cherry tree leaves, DMD: dry matter digestibility, DMI: dry matter intake, RFV: relative feed value, IVTD: *in vitro* true digestibility, SL: significant level. In determining forage quality, an RFV score of "5" indicates very poor quality that is unsuitable for use, while "1" represents good quality, and "prime" denotes the highest quality standard [7].

The *in vitro* gas production, gas production parameters, methane production, energy values, and organic matter digestibility of vegetable harvest residues and tree leaves are presented in Tables 6 and 7. According to the results, the additives added to vegetable harvest residues showed no significant effect during the 24-hour incubation period. However, the gas production values determined for tomato residues were significantly lower than those for pepper and eggplant residues (P<0.05), with no statistical difference observed between pepper and eggplant residues. For all residues, the addition of additives tended to reduce gas production numerically. Regarding the *in vitro* gas production rate "c value," the use of additives had no significant impact, although pepper residues showed the highest c value. No differences in gas production rate were found between tomato and eggplant residues (P>0.05). Similarly, adding additives did not significantly affect the total gas production. Methane production was lowest in groups containing tomato residues (P<0.05), while no significant differences in methane production were observed among the other groups (P>0.05).

The organic matter digestibility (OMD), metabolizable energy (ME), and net energy lactation (NE_L) contents were lowest in the groups containing tomato residues among the vegetable harvest residues. Additive inclusion did not significantly affect these parameters across all residues (P>0.05). For tree leaves, similar to other incubation periods, hazelnut leaves (HTL) showed the lowest values during the 24-hour incubation. Hazelnut leaves also exhibited the lowest values for gas production rate and total gas production (a+b), while fig leaves (FTL) had the highest gas production rate. Methane production was similar between fig leaves and black cherry leaves (CTL), but hazelnut leaves had the lowest methane production. Fig leaves exhibited the highest OMD, ME, and NEL values among the gas production parameters, while hazelnut leaves displayed the lowest values. Black cherry leaves were intermediate, with significant differences among all tree leaves for these parameters (P<0.05). Mahmoud et al. [18] strongly recommend the practical use of pepper and eggplant harvest residues as a 12.5% replacement for clover in the diets of dairy cows.

Table 6 *In vitro* gas production (ml/200 mg DM), gas production parameters, and pH after 96 h incubation of some vegetable harvest residues

Treatments	3	12	24	96	c, ml/h	a+b, ml	Methane, ml	OMD %	ME	NE_L	pH
TSL	7.39±0.29c	19.22±1.14b	23.66±2.18bc	30.23±2.03cd	0.09±0.01b	28.19±2.10de	5.51±0.53b	47.12±1.84c	5.96±0.28c	2.79±0.21c	6.90±0.05a
TSLM	6.84±0.65c	19.47±1.22b	22.80±2.03c	29.65±2.20cd	0.09±0.00b	27.91±2.16de	4.91±0.08b	47.19±1.80c	6.05±0.27c	2.82±0.21c	6.82±0.03abc
TSLE	6.24±1.07c	16.51±2.63b	20.13±1.83c	25.30±1.74d	0.13±0.03b	22.75±1.89e	4.75±0.58b	44.46±1.63c	5.73±0.26c	2.55±0.19c	6.71±0.03c
ESL	12.06±0.41a	26.52±1.32a	31.22±1.86a	38.41±1.67a	0.08±0.01b	36.79±1.59a	8.09±0.69a	59.03±1.68ab	8.59±0.26a	4.29±0.19a	6.77±0.02bc
ESLM	11.43±0.45ab	26.13±0.65a	31.20±1.40a	38.37±1.84a	0.11±0.02b	35.94±1.73ab	8.09±0.75a	59.93±1.19ab	8.82±0.17a	4.29±0.13a	6.84±0.03ab
ESLE	11.33±0.49ab	26.15±1.23a	31.43±2.01a	36.63±2.33ab	0.13±0.02b	34.92±2.49abc	8.08±0.80a	60.53±1.75a	8.91±0.26a	4.31±0.20a	6.80±0.03abc
PSL	11.92±0.65a	29.71±1.51a	30.65±1.76a	34.14±1.75abc	0.22±0.01a	32.26±1.58abcd	8.01±0.47a	55.46±1.55ab	7.37±0.24b	3.82±0.18ab	6.72±0.04c
PSLM	9.73±0.73b	28.49±2.15a	28.75±1.95ab	31.82±2.39bc	0.21±0.02a	30.27±2.29bcd	7.64±0.63a	54.78±1.71b	7.33±0.26b	3.61±0.20b	6.76±0.03bc
PSLE	11.14±0.68ab	26.99±1.32a	28.93±1.62ab	30.37±2.01cd	0.21±0.02a	29.53±1.74cd	7.32±0.61a	54.84±1.44b	7.28±0.22b	3.69±0.17b	6.81±0.04abc
SL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016

$P<0.05$; a,b…, differences between means with different letters in the same column are significant. TSL: tomato stems and leaves, TSLM: tomato stems and leaves with molasses addition, TSLE: tomato stems and leaves with ecomass addition, ESL: eggplant stems and leaves, ESLM: eggplant stems and leaves with molasses addition, ESLE: eggplant stems and leaves with ecomass addition, PSL: pepper stems and leaves, PSLM: eggplant stems and leaves with molasses addition, PSLE eggplant stems and leaves with ecomass addition, c: gas production rate, a+b: total gas production, OMD: organic matter digestibility, ME: metabolizable energy, NE_L: net energy lactation, pH: pH measured after 96 hours of incubation, SL: significant level.

Table 7 *In vitro* gas production (ml/200 mg DM), gas production parameters, and pH after 96 h incubation of some tree leaves

Leaves	3	12	24	96	c, ml/h	a+b, ml	Methane, ml	OMD %	ME	NE_L	pH
FTL	14.88±0.83a	32.47±1.45a	37.57±1.60a	44.97±1.47a	0.10±0.01a	42.92±1.46a	8.76±0.46a	65.30±1.42a	8.93±0.22a	5.01±0.17a	6.60±0.04
HTL	7.99±0.76b	13.55±1.05c	15.91±1.89b	21.89±2.15b	0.04±0.00c	21.91±2.06b	3.62±0.45b	40.79±1.67c	5.69±0.26c	2.72±0.19c	6.60±0.04
CTL	9.40±0.81b	25.91±1.65b	34.05±1.71a	42.18±1.45a	0.08±0.01b	41.03±1.39a	7.48±0.53a	55.69±1.51b	8.15±0.23b	4.27±0.16b	6.57±0.03
SL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.871

$P<0.05$; a,b…, differences between means with different letters in the same column are significant. FTL: fig tree leaves, HTL: hazelnut tree leaves, CTL: cherry tree leaves, c: gas production rate, a+b: total gas production, OMD: organic matter digestibility, ME: metabolizable energy, NE_L: net energy lactation, pH: pH measured after 96 hours of incubation, SL: significant level.

4. Conclusion

According to the findings, incorporating vegetable residues into rations as a secondary roughage source, rather than using them as a sole roughage source in animal feeding, appears more appropriate. This approach could significantly contribute to reducing methane production. However, it should be noted that low-quality roughages generally lead to higher methane production. Therefore, combining these residues with low-quality roughages may also be feasible. Among the tree leaves evaluated, fig leaves have a higher roughage potential than the others. It is also important to conduct studies on the evaluation of tree leaves and vegetable residues in animal feeding using different additives. It is recommended that future studies be planned to examine different additives in the materials in question and to evaluate them as silage additives (to prevent the breakdown of proteins) or by adding them to concentrated or roughage feeds at specific rates. As a result, it is recommended that especially hazelnut leaves and tomato residues have a significant effect on reducing methane production, that the nutrient contents and nutritional values of the vegetable residues and tree leaves used in the study are revealed, and that the additives added to the vegetable residues are repeated using different doses and different additives.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

Authors' Contribution

Hasret CELIK contributed to the procurement of feed materials, preparation for analysis, and execution of analyses and provided support during the writing phase. Unal KILIC contributed to the study's design, conducted analyses, and assisted in the writing phase.

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