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Potential feeding value, feed quality, microbial protein production, and anti-methanogenic properties of tree leaves admixed with Italian ryegrass at different ratios using *in vitro* gas production

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Abstract

The purpose of this study was to use the *in vitro* gas production (IVGP) method to assess the relative feeding value, feed quality, and anti-methanogenic capabilities of tree leaves added to Italian ryegrass forage at various proportions. With the addition of 2% and 4% willow leaf (*Salix alba*), 2% and 4% vine leaf (*Vitis vinifera*), 2% and 4% plane leaf (*Platanus orientalis*), and a control group devoid of these supplements, a total of 21 forage samples were created. The data were subjected to analysis of variance after nutritional composition, energy content, feeding value, and feed quality were determined using the *in vitro* gas generation method. The effect of the addition of tree leaves to forage on crude ash, IVGP, methane, metabolizable energy, net energy lactation, organic matter digestion, true digestible dry matter, partitioning factor, microbial protein, microbial protein synthesising efficiency, degree of true digestion, NDF digestion, total digestible nutrients, and relative feed quality was substantial. With the addition of 2% willow leaves, the digestibility, metabolic energy, net energy lactation value, organic matter digestion, microbial protein value, and relative feed quality all improved. The findings showed that tree leaves can be utilized as an alternate feed source for ruminants. Tannin content should be taken into account when preparing rations utilizing tannin-containing tree leaves.

Keywords: **w**illow, vine, plane, *in vitro* digestibility, anti-methanogenic, feed quality
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Introduction

Roughages must be included in rations to ensure that ruminants are fed properly. The digestive system benefits from roughages, which assist in the meeting of the protein and caloric requirements of the animal. Intestinal health, intestinal flora, and higher feed intake are further advantages. As a result, the quality of the roughage plays a crucial role in the nutrition of ruminant animals (Sing *et al*., 2022; Zhu *et al*., 2022). Italian ryegrass, which is now often farmed, has gained popularity as a feed for cattle, goats, sheep, and other ruminants in recent years. Due to its low lignin content and high digestion rate, this plant is now commonly used for feeding ruminants. Italian ryegrass is a great feed for livestock raised for meat output as well as for animals with excellent milk yields. The high protein and energy content of Italian ryegrass makes it an excellent source of nutrients for animals (Gayer *et al*., 2019).The shortage of high-quality roughage may be compensated for by alternative feed items like tree leaves. In the absence of high-quality roughage, tree leaves can be utilized as an alternative to give animals the nutrients they require. Additionally, the cost of tree leaves is low. Tree leaves can be used to meet the roughage requirement when there are not any new grasses or other green plants, especially in the winter (Simbaya *et al*., 2020; Karan & Basbag, 2022).

In ruminant animals, willow leaves provide a premium source of nutrition. Willow leaves are a rich source of protein and dry matter for animal feeds and they are highly digestible. Additionally, ruminants eating willow leaves produce less methane. Owing to the high tannin content of willow leaves, overusing them may result in gastric issues; therefore, willow leaves should be used responsibly and in a balanced way. The immune system can be supported by willow leaf, which also contains antibacterial

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and antioxidant characteristics. Willow leaf can therefore aid in the prevention of several diseases (Seidavi *et al*., 2020).

Vine leaves are distinguished by their high content of protein and calories. They also have substantial amounts of polyphenols and flavonoids, which are antioxidants. These substances are crucial for the wellbeing and productivity of animals. They are also advantageous for the health of the digestive system. Grapevine leaves increase the activity of digestive enzymes, which enhances digestive system activities and supports intestinal health (Peiretti & Tassone, 2019; Kazemi & Bezdi, 2022). However, they might prevent ruminants from digesting protein and might lead to digestive issues in animals because they contain tannins. As a result, vine leaves should be added to rations with care and in a balanced manner with other feed sources (Birkinshaw *et al*., 2022).

The high protein and calcium content of sycamore leaves makes them a valuable feed source, particularly for dairy calves. The huge amount of fibre in this plant is also an advantage. As a result, it is a crucial feed source to guarantee the healthy operation of ruminant digestive systems. Additionally, ruminant digestive health can be maintained, and the overall health of the animal benefits from the tannins in plane leaves. However, excessive use of plane leaves can result in kidney stone production because they contain high levels of oxalic acid. It is important to balance the usage of plane leaves with other feed sources and limit intake (Tesfay *et al*., 2018).

Feeding value for ruminants is based on the bioavailability of nutrients in feeds and their impact on animal growth, development, milk yield, and other production characteristics. Feed quality refers to the nutrient-related properties of feed and their availability to animals (Tassone *et al*., 2022).The ability of animal feeds to produce methane is tested using *in vitro* methane generation. In order to mitigate the environmental effects of the methane produced by animal digestive processes, this technique is a crucial tool in the selection and use of animal feed. In the assessment of attempts to minimize the methane gas produced by ruminant animals, which contributes to global greenhouse gas emissions, methane production from feed is important. Bovine and ovine ruminant anaerobic digestive systems produce the gas methane, which is then discharged into the atmosphere. To ascertain the steps to be taken to reduce bovine greenhouse gas emissions, it is crucial to ascertain the methane generation capacity of feeds used in feeding ruminants. The ability of feeds to produce methane is correlated with the digestibility, metabolic energy content, and protein value of feeds (Kazemi & Bezdi, 2021; Liu *et al*., 2022). Therefore, the methane production potential of feeds is important for optimising the nutrition and performance of ruminants

The study is important for the reasons that follow. The plants examined in this study (willow, vine, and pine tree leaves) are typically regarded as waste. However, this study demonstrates that these plants can be used to produce animal feed, improving sustainability in the industry. The study demonstrates that the addition of willow, grapevine, and pine tree leaves to animal feed helps to raise the metabolic energy of animals and increase microbial protein production. This improves animal health and performance. Methane, one of the greenhouse gases, is a significant consequence of the manufacture of animal feed. According to this study, adding leaves from willow, vines, and pine trees to animal feed can help lower methane emissions and mitigate environmental effects. This research promotes the use of novel materials in the feed industry. Willow, vine, and pine tree leaves are examples of plants that are typically regarded as waste. However, this study demonstrates that these plants can be used to make animal feed, encouraging innovation in the feed industry.

The purpose of this study was to ascertain how adding willow, vine, and pine tree leaves to Italian rye grass at various rates affected its chemical composition, *in vitro* gas production, methane production, metabolic energy, true digestibility of organic matter, microbial protein production, microbial protein synthesis effect, feeding value, and feed quality.

Materials and Methods

The Italian ryegrass and the willow, vine and plane tree leaves were harvested from a field in Erzincan, Turkey; leaves from at least 10 different trees were harvested in June 2022 and brought to the laboratory. The samples were dried in the laboratory at 105 °C in an oven for 24 h and used in the analysis. After drying, the leaves were ground with a 1-mm sieve mill. For the 2% and 4% willow, vine, and plane leaf supplementation and a control group devoid of supplementation, groups with three duplicates were created. Dry matter (DM), crude ash (CA), and crude protein (CP) contents of the samples (three replicates) were determined according to the methods of the AOAC (1990); condensed tannin (CT) content was determined according to the method of Makkar *et al*. (1995); and acid detergent fibre (ADF) and neutral detergent fibre (NDF) content of the samples was determined according to the method of Van Soest *et al*. (1991) (Table 1).

Table 1 Nutrient composition of Italian ryegrass and willow, vine, and plane leaves (%)

Feeds	DM	CA	CP	ADF	NDF	CT
Italian ryegrass	94.00	10.32	20.66	24.43	48.18	0.45
Willow leaves	92.57	10.24	22.83	28.61	63.67	5.57
Vine leaves	93.28	8.14	20.70	19.94	36.35	5.22
Plane leaves	94.17	6.16	16.22	36.60	52.40	9.93

Dry matter (DM), crude ash (CA), crude protein (CP), condensed tannin (CT), acid detergent fibre (ADF), neutral detergent fibre (NDF)

Relative feeding value (RFV) was calculated using the formula developed by Van Dyke & Anderson (2000) from the estimation of the energy value of feeds based on their potential consumption by animals:

%DMD (Dry matter digestion) = 88.9 - (0.779 × %ADF) (1)
%DMI (Dry matter intake) = 120 / NDF (2)
RFV = %DMD × %DMI × 0.775 (3)

The buffer solutions required for the determination of *in vitro* digestibility parameters using the Ankom Daisy incubator were prepared as required by the Ankom Daisy *in vitro* fermentation system user manual. After 48 h, true dry matter digestion (TDMD), true NDF digestion (TNDFD), true organic matter digestibility (TOMD), DMI, and total digestible nutrient (TDN) values were obtained in the Daisy incubator and the relative feed quality (RFQ) was determined using the following equation:

RFQ = (DMI, % DM) × (TDN, % DM) / 1.23 (Ward and Ondarza, 2008) (4)

Metabolic energy (ME) and net energy lactation (NE_L) values of feedstuffs were determined using the equation of Menke & Steingass (1988):

OMD (organic matter digestibility), % = 15.38 + 0.8453×GP + 0.0595×CP + 0.0675×CA (5)
ME, MJ/kg DM = 2.20+0.1357×GP + 0.057×HP + 0.002859 ×HY^2 (6)
NEL (MJ/kg DM) = 0.101×GP + 0.051×CP + 0.112×CF (7)

where GP, net gas production of 200 mg dry feed sample at the end of 24 h incubation period; CP, % crude protein; CF: % crude fat and CA: % crude ash).

Three adult, female Montofon cattle slaughtered at the Meat and Fish Institution in Erzurum and whose slaughter had been authorized by the Ethics Committee had their rumen contents collected. The rumen of each animal was opened within 5 min of slaughter and the liquid was taken from the rumen and placed in a thermos previously brought to 39 ºC; CO_2 was added, and the flask was transported to the feed analysis laboratory. Approximately 500 mg of the feed samples was incubated for 24 h in a water bath at 39 ˚C in 100 ml glass syringes together with 40 ml of rumen fluid with buffer solution (Menke *et al*., 1979). After 24 h of fermentation, the amount of methane (%) in the total gas produced was determined using an Infrared Methane Analyser (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008). After the gas measurements, the rumen fluid and feed samples remaining in the syringe were boiled in NDF solution prepared as reported by VanSoest *et al*. (1991) for 1 h. After the boiling process, the true digested dry matter content, true digestion degree, partition factor, microbial protein production, and synthesis efficiency values were determined using the equations of Blümmel *et al*. (1997):

DTD (degree of true digestion) (%) = ((Incubated DM (mg) - Remaining DM (mg))/ Incubated DM (mg)) ×100 (8)
TDDM (true digestible dry matter) (mg) = Incubated DM (mg) – Remaining DM (mg) (9)
Partition Factor (PF)= TDDM/GP (gas production) (10)
Microbial recovery MP (mg) = TDDM – (GP (24 h) × 2.2 mg/ml)) (11)
Microbial Protein (MP) (mg/g DM) = TDDM – (GP × 2.2 mg/ml), (12)
Microbial Protein Synthesising Efficiency (MPSE) = (TDDM – (GP × 2.2 mg/ml))/TDDM (13)

Gas and methane production of tree leaves are presented in Table 2.

Table 2 *In vitro* gas production (IVG), methane production, ME, NE_L, and OMD values of willow leaves, vine leaves, and plane leaves

Feeds	**IVG** %	**Methane** (ml)	**Methane** (%)	**ME** MJ/kg DM	**NE_L,** MJ/kg DM	**OMD** %
Willow Leaf	69.39	8.59	12.41	7.27	4.07	56.46
Vine Leaf	95.45	14.08	14.78	8.56	4.99	63.41
Plane Leaf	53.37	7.24	13.58	6.02	3.20	45.14

Metabolizable energy (ME), net energy lactation (NE_L), organic matter digestibility (OMD)

The data were subjected to analysis of variance in SPSS 24 (IBM, 2016) and Duncan's comparison tests were applied to compare the groups.

Results

The effect of different ratios of tree leaves added to Italian ryegrass on nutrient composition is shown in Table 3. The addition of tree leaves (2, 4% willow leaves; 2, 4% vine leaves; 2, 4% plane leaves) to Italian ryegrass caused differences in the CA (P <0.05). The CA content, which was 10.24% in the control group, decreased in the willow leaf group (2, 4%), while it tended to increase in the vine and plane leaf groups (2, 4%). In the willow leaf groups, the CA, which was 10.24% in the control group, fell (2, 4%), but tended to increase (2, 4%) in the vine and plane leaf groups.

Table 3 The effect of different ratios of tree leaves on nutrient composition of Italian ryegrass, %

Feeds	**DM**	**CA**	**CP**	**ADF**	**NDF**
Italian Ryegrass	94.00	10.32^{bc}	20.66	24.43	48.18
2% Willow Leaf	94.21	9.42^{d}	20.13	21.94	49.36
4% Willow Leaf	94.21	9.60^{cd}	20.23	24.15	51.29
2% Vine Leaf	94.04	11.58^{a}	19.78	26.02	50.88
4% Vine Leaf	94.13	11.01ab	20.27	24.41	49.43
2% Plane Leaf	95.08	11.73^{a}	22.12	25.14	51.92
4% Plane Leaf	94.36	10.63^{b}	20.80	26.16	49.90
SEM	0.12	0.20	0.25	0.40	0.44
P	0.258	0.000	0.235	0.075	0.321

^{a,b} Differences between means with different letters in the same column are significantly different, P <0.05; DM: dry matter, CA: crude ash, CP: crude protein, NDF: neutral detergent insoluble fibre, ADF: acid detergent insoluble fibre, ADL: acid detergent insoluble lignin, SEM: standard error of mean, P: significance level

The mean IVGP and methane production, ME, NE_L, and OMD values and the ANOVA results of the different tree leaf inclusions to Italian ryegrass are shown in Table 4. There were differences (P <0.05) in the parameters of IVGP, methane (ml), ME, NE_L, and OMD values of all tree leaves (2, 4% willow, vine, and plane leaves) to Italian ryegrass. The tree leaves (except 2% willow) decreased the IVG production of the feed. Willow, vine, and plane leaves at 4% decreased the methane production (ml); the highest decrease was observed in 4% willow leaves (17.7 ml). The group with 2% willow leaves had the highest methane (ml) production. In the 2% willow leaf group, ME, NE_L, and OMD values increased the most (10.00, 6.00, and 73.58). The other tree leaf additions resulted in a decrease in these parameters.

Table 4. Average gas and methane production, ME, NE_L, and OMD values and results of the analysis of variance for different proportions of tree leaves added to Italian ryegrass

Feeds	IVGP %	Methane (ml)	Methane (%)	ME MJ/kg DM	NE_L MJ/kg DM	OMD %
Italian Ryegrass	118.63^{ab}	18.97^{abc}	16.04	9.82^{ab}	5.87^{ab}	73.06^{a}
2% Willow Leaf	122.43^{a}	20.87^{a}	17.09	10.00^{a}	6.00^{a}	73.58^{a}
4% Willow Leaf	108.45^{c}	17.70^{c}	16.34	9.24^{c}	5.47^{c}	68.77^{b}
2% Vine Leaf	117.21^{ab}	19.61^{abc}	16.77	9.69^{ab}	5.79^{ab}	72.97^{a}
4% Vine Leaf	114.72^{abc}	18.54^{bc}	16.20	9.58^{ab}	5.71^{abc}	71.94^{a}
2% Plane Leaf	115.67^{abc}	19.69^{ab}	17.08	9.74^{ab}	5.82^{ab}	73.57^{a}
4% Plane Leaf	112.55^{bc}	17.80^{bc}	15.86	9.50^{bc}	5.65^{bc}	71.15^{ab}
SEM	1.20	0.29	0.14	0.30	0.04	0.41
P	0.028	0.021	0.111	0.032	0.032	0.027

a, b, c Means with different symbols in the same column are different from each other (P <0.05); IVGP: 24-hour *in vitro* gas production, ME: Metabolic energy, NE_L: Net energy lactation, OMD: organic matter digestibility, SEM: standard error mean, P: significance level

The effects of different additions of tree leaves on TDDM, PF, MP, MPSE, and DTD values and the ANOVA are shown in Table 5. Tree leaves (2, 4% willow vine, and plane leaves) added to Italian ryegrass caused differences in TDDM, PF, MP, MPSE, and DTD (P <0.05). The 2% willow leaf group (295.56, 2.77, 61.24, 61.24, 20.50 and 58.77%) had higher TDDM, PF, MP, MPSE, and DTD values. The highest decrease in TDDM and DTD values took place with 4% vine leaves (226.15 and 44.73).

Table 5. TDDM, PF, MP, MPSE, and DTD values and analysis of variance for different proportions of tree leaves added to Italian ryegrass

Feeds	TDDM	PF	MP	MPSE	DTD
Italian Ryegrass	230.80^{c}	2.23^{b}	3.76^{b}	1.61^{c}	46.66^{d}
2% Willow Leaf	295.56^{a}	2.77^{a}	61.24^{a}	20.50^{a}	58.77^{a}
4% Willow Leaf	239.69^{bc}	2.56^{ab}	32.13^{ab}	12.99^{ab}	47.95^{cd}
2% Vine Leaf	258.37^{b}	2.53^{ab}	34.04^{ab}	13.08^{ab}	52.33^{b}
4% Vine Leaf	226.15^{c}	2.26^{b}	6.59^{b}	2.91^{bc}	44.73^{d}
2% Plane Leaf	254.07^{b}	2.52^{ab}	32.71^{ab}	12.54^{ab}	51.33^{bc}
4% Plane Leaf	236.37^{bc}	2.41^{b}	20.97^{b}	8.85^{bc}	48.17^{cd}
SEM	5.35	0.05	4.98	1.70	1.03
P	0.000	0.030	0.011	0.016	0.000

a, b, c, d Means with different symbols in the same column are different from each other (P <0.05); TDDM: true digestible dry matter, PF: partition factor, MP: microbial protein (mg), MPSE: microbial protein synthesis efficiency, DTD: degree true of digestion, SEM: standard error of mean, P: significance level

The RFV, NDFD, TDN, DMI, and RFQ values and ANOVA results from different proportions of tree leaves added to Italian ryegrass are shown in Table 6. Tree leaves (2, 4% willow, vine, plane leaves) added to Italian ryegrass caused differences in NDFD, TDN, and RFQ (except RFV and DMI) (P <0.05). RFV was decreased proportionally by tree leaf addition. Willow leaves (58.22, 59.44, and 117.46%) increased NDFD, TDN, and RFQ values, respectively, the most. Relative to the control group, the values of NDFD, TDN, and RFQ decreased with the addition of tree leaves.

Table 6. RFV, NDFD, TDN, DMI, and RFQ values and analysis of variance results of the addition of 2% and 4% tree leaves to Italian ryegrass

Feeds	RFV	NDFD	TDN	DMI	RFQ
Italian Ryegrass	135.01		55.97^{b}	2.49	113.42^{ab}
2% Willow Leaf	135.42	58.22^{a}	59.44^{a}	2.43	117.46^{a}
4% Willow Leaf	127.27	50.16^{bc}	55.98^{b}	2.34	106.57^{bc}
2% Vine Leaf	125.88	55.65^{ab}	55.42^{bc}	2.36	106.65^{bc}
4% Vine Leaf	131.87	47.52^{c}	53.66^{c}	2.43	106.25^{bc}
2% Plane Leaf	124.21	53.98^{abc}	54.39^{bc}	2.31	102.21^{c}
4% Plane Leaf	127.76	49.99^{bc}	55.21^{bc}	2.40	107.97^{abc}
SEM	1.58	1.04	0.43	0.02	1.45
P	0.363	0.021	0.01	0.319	0.067

[a,b,c] Differences between means indicated by different letters in the same column are different, *P* <0.05; RFV: relative feeding value, NDFD: NDF digestibility, TDN: total digestible nutrient, DMI: dry matter intake, RFQ: relative feed quality, SEM: standard error mean, P: significance level

Discussion

Only the CA content was statistically altered by the addition of tree leaves to Italian ryegrass; it was increased by vine and plane leaf and lowered by willow leaf addition. This might be as a result of the ingredients in willow leaves being more potent than those in other tree leaves or because some of the ingredients in willow leaves are unique from those in other leaves. In addition, the amount and composition of the added leaves may also influence these differences. The changes in CA concentration between tree species may have resulted from both internal and external factors, such as mineral composition of the scrublands. Such factors are associated with variations in the mineral content of the soil (Unlu *et al*., 2022). Similarly, Oruç (2016) reported that the addition of willow leaves to wheat straw reduced the CA content more than the control group. Cengiz and Kamalak (2020) reported CA values of willow leaves collected from different provinces of 10.45–13.27%. Kurt (2022) found the CA values of tree leaves were 6.21–11.78% and that there was a statistical difference between the groups, whereas Unlu *et al*. (2022) stated that the CA content of different tree leaves differed from each other, which is consistent with the finding in the current experiment.

According to studies using the *in vitro* gas method, condensed tannins in tree leaves have an impact on how quickly the rumen microorganisms break down the feed, which creates an inverse relationship with gas production from fermentation (Ku-Vera *et al*., 2020). The current study showed that the IVGP decreased as the percentage of willow, vine, and plane leaves increased. According to the method developed by Lopez *et al*. (2010), the methane gas values formed in feeds can be classified as low anti-methanogenic (>11% and 14%), medium anti-methanogenic (>6% and 11%), and high anti-methanogenic (>0% and 6%). The preparation of rations that consider these classifications of feeds can improve the efficiency of energy use in ruminants to reduce the methane gas that causes global warming. No anti-methanogenic effect was seen in any of the ration groups that were given tree leaves in this study. The components in willow and plane leaves may depress methane production, which may account for the largest decrease in methane (ml) production with 4% willow and 4% plane leaf supplementation. Compounds such as tannins, saponins, phenolic acids, and flavonoids in these plant species reduce methane production in ruminants. In addition, the high fibre content in willow and plane leaves may also reduce methane production by reducing microbial activity. The compounds and fibre content of grapevine leaves may have a different effect to willow and plane leaves. The differences between the *in vitro* gas production of tree leaves may be due to the chemical composition of tree leaves, vegetative state of leaves, and soil structure. Özdemir & Kaya (2020) stated that the methane values of different tree leaves varied between 14.07–15.53% and there was a statistical difference between the groups. Tree leaves from different species produce gas and methane *in vitro* in different ways due to varying chemical and fibre compositions. Differences in *in vitro* gas generation may also be caused by factors such as vegetative condition, soil structure, and chemical makeup of tree leaves. Therefore, it is important to consider the content and effects of tree leaves before using them in animal feeding trials.

The highest increases in ME, NE_L, and OMD in the feed were observed with 2% willow leaves. Of the factors affecting the nutritional values in feeds are the composition, properties, and digestibility of the leaves. Willow leaf has a high digestibility due to its high protein and low lignin content. High protein content can increase microbial activity, resulting in more microbial protein synthesis in ruminants. Therefore, the addition of 2% willow leaves can increase the digestive and metabolic energy values of the feed and increase the NE_L. The values obtained for ME, NE_L, and OMD were found to be higher than those put forward by other researchers (El-Waziry *et al*., 2015; Kaya *et al*., 2022; Unlu *et al*., 2022). Oruç & Avcı (2018) determined the ME level of willow leaf as 8.78–8.95 MJ/kg DM. Özdemir & Kaya (2020) stated that the ME values of different tree leaves varied from 4.61–7.03 MJ/kg DM, while the ME value of willow leaf was 5.41 MJ/kg DM and there was a statistical difference between the groups. The same researchers found the NEL value of willow leaves to be 3.93 MJ/kg DM, which was lower than the values obtained in the experiment (5.47–6.00 MJ/kg DM). Cengiz & Kamalak (2020) stated that the ME values of willow leaves collected from different provinces were 6.91–8.18 MJ/kg DM. These values were lower than the values obtained in the current experiment. The nutritional values are impacted by changes in the content of the feed, particularly by elements like digestibility, composition, and qualities of the plants added to the feed. As a result, the nutritional content of feeds can be substantially impacted by the characteristics of plants added to them, such as willow leaves. Willow leaf can improve feed digestion and metabolizable energy values since it has a high digestibility because of its high protein and low lignin content. Plant components may differ in nutritional value depending on the region where the plants are grown, the soil conditions, the age of the plant, and the time of harvest. As a result, there can be variations in the nutritional values found in various studies. Environmental elements including the region where the plant is cultivated, the time of harvest, and the soil conditions may be the cause of these variations.

Determination of the partition factor, microbial protein, and microbial protein synthesis in feeds is important to help meet the nutritional needs of animals accurately. The partition factor is a factor that determines the digestibility of feeds. This factor measures the effectiveness of the crude protein in the feed in meeting the available protein requirement in the animals' body. The allocation factor is used in the evaluation of feeds and helps to determine the amount of feed to meet the protein needs of animals. Microbial protein occurs during the fermentation of feeds in ruminants. It is synthesised by microorganisms and digested by animals. Microbial protein plays an important role in meeting the body protein requirement of animals and is essential for animals to be highly productive. Microbial protein synthesis is a method for determining the amount of microbial protein produced during fermentation of feeds in ruminants. This method helps to determine the amount of feed that will meet the protein needs of animals. Determination of these factors helps to prepare a correct feeding programme to meet the nutritional needs of animals. This helps animals to grow, develop, and produce efficiently (Baba *et al*., 2002; Abd’quadri-Abojukoro & Nsahlai, 2023). The measured values can be impacted by the amount of protein, starch, and fat in the feed mix. A high protein concentration can boost microbial activity, which in turn can boost microbial protein synthesis and partition factor values. Feeds with high digestibility have the potential to boost metabolic and digestive energy levels, while feeds with low digestibility have the potential to lower these levels. The measured parameters are also impacted by fermentation time. In contrast to short fermentation times, long fermentation times can result in high microbial protein synthesis and partition factor values.

Willow leaf is characterised by higher TDDM, PF, MP, MPSE, and DTD values, compared to other leaf types. These results indicate that leaves such as willow leaves are more effective in increasing the nutritive values of feed. Therefore, if willow leaf is added to the feed, the nutritive values of the feed will increase and help animals to fulfil their nutritional requirements. The addition of vine leaves to the feed caused the highest decrease in the TDDM and DTD values of the feed. This result indicates that vine leaf decreases the nutritive values of the feed and therefore animals need to eat more feed to meet their nutritional requirements. It has been reported that roughages with high PF values have high digestibility and feed intake (Blümmel *et al*., 1997; Baba *et al*., 2002). Cengiz & Kamalak (2020) reported that PF values of willow leaves collected from different provinces were 4.04–4.69%. These values were higher than the values obtained in the current experiment. The composition of feed ingredients and how they interact with the digestive system may be the cause of effect on the observed parameters. For instance, because willow leaf has less cellulose and lignin, it has high TDDM, PF, MP, MPSE, and DTD values. In the digestive tract of animals, high-fibre components like cellulose and lignin are less digestible and produce less energy. As feeds with high PF values have a high level of feed intake and digestibility, their nutritional content may be higher. The components in vine leaves may not be compatible with the digestive systems of animals, which would explain why they lower the nutritious content of feed. Vine leaves contain tannins, which can bind to proteins and hinder digestion. As a result, adding vine leaves to feed may make it harder for animals to digest the protein, forcing them to consume more feed to meet their protein needs. The impact of environmental variables in the various geographic regions where willow leaf samples were gathered can be used to explain why the PF values in willow leaves were higher than those measured in the experiment. For instance, changes in the nutritional content of plants may be caused by soil characteristics, environmental factors, and plant cultivation techniques.

Feed quality depends on the protein, energy, fibre, vitamin and mineral content, digestibility, non-toxicity, freedom from foreign substances, chewability, and palatability. The Daisy Incubator is a test method used to determine the digestibility of ruminant feeds. This test method is simpler, cheaper, and faster than other methods such as the *in vitro* gas production test (IVGP), which is another test method used to determine feed quality. In the Daisy Incubator test, the feed sample is digested by microorganisms in the incubator and its digestibility is determined by measuring the gases accumulated in the incubator (Tassone *et al*., 2022). The 2% willow leaf addition increased the NDFD, TDN, and RFQ values substantially. Different tree species have different effects on forage quality and the digestibility of Italian ryegrass. It was also observed that willow leaves provided a higher feed quality and digestibility compared to other tree species. This may be due to the high protein and low cellulose content of willow leaves. Lower feeding value in tree leaves may be due to their high fibre content. ADF, NDF, and HS values were examined in a study with plane leaves, and it was shown that low usefulness in the ration was related to their high cellulose content, low crude protein content, high dry matter digestibility, and relative feeding values (Pala, 2023). Özdemir & Kaya (2020) found that the RFV of willow leaf was 182.48, which was higher than the values obtained in the current trial (127.27–135.42). The variations in the feed sample constituents and how these constituents interact with the digestive system may be the cause of this discrepancy. High protein and low cellulose content in the willow leaf sample may improve the quality and digestibility of the feed. High protein content may be effective in meeting the protein requirement of animals and increase microbial protein synthesis. Low cellulose concentration can increase the ability of animals to digest feed and increase nutritional absorption. Characteristics of the sycamore leaf sample, such as its high cellulose content, low crude protein values, and poor dry matter digestibility rate, could have a detrimental impact on the quality and digestibility of feed. Animals may have trouble digesting and utilizing nutrients because of high cellulose content. The nutrient composition of the plants determines the impact of the tree species. Each plant species has a unique nutrient profile, which may cause them to have distinct effects on feed quality and digestibility.

Conclusions

The study investigated the addition of tree leaves to Italian ryegrass on the quality of the feed as well as how much methane was produced by ruminants. It was found that while the type and quantity of additional leaves could impact the nutrient composition and ability to produce methane, they were not anti-methanogenic. Willow and plane leaves increased the nutritional value of the feed and decreased methane production. Tannins, saponins, phenolic acids, and flavonoids in willow and plane leaves can reduce methane production. Willow leaf supplementation (2%) increased the digestibility, ME, NE_L, and relative feed quality of the feed. In conclusion, the effects of adding different types of leaves to the diet depends on their composition and digestibility, and further research is needed to determine the optimal amounts and combinations of leaves to achieve the desired nutritional and environmental outcomes.

Authors' Contributions

The authors declare that they have contributed equally to the study.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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