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Research article

Determination of nutritional value and methane production potential of styrax tree (*Styrax officinalis L.*) leaves

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ABSTRACT

This study aimed to determine the effect of nutritional values of the “styrax tree” (Styrax officinalis L.) on in vitro gas production, organic matter digestibility (OMD), and metabolic energy (ME) content. In this study, styrax leaf samples were collected for analysis in three different periods: May, July and September. According to the analysis results, crude ash (CA), dry matter (DM), crude fat (CF), acid detergent fiber (ADF) values, and neutral detergent fiber (NDF) increased in September compared to May and July. In September, compared to May, CA has increased by 23 %, DM by 64 %, CM by 30.5 %, ADF by 9.8 %, and NDF by 9.8 %. During the vegetation period, there was an increase in dry matter content in parallel with maturation. The dry matter content of Styrax tree leaves (STL) increased in May (30 %), July (40 %) and September (50 %). Crude protein amounts were found 19 % in May, 17 % in July, and 15 % in September. As the STL grows, decreases in CP, which plays a role in plant growth, and increases in the cell membrane were observed. The CF content in STL changed to 3.2 %, 4.4 %, and 4.3 % in May, July, and September, respectively, with the highest value determined in July and very close to the September value. CA content in styrax tree plants varied by 8.58 % in May, in July by 9.86 %, and in September by 10.56 %. As the amount of dry matter increased according to the periods, the amount of CA also increased. NDF and ADF levels increased in parallel with the vegetation period’s development in STL. NDF was found to be 37.5 %, 39.0 %, and 41.5 % according to the periods. ADF amount was found 27 % in May, 28.5 % in July, and 30 % in September. Metabolic energy varied between 8.86 and 10.06 (MJ/kg KM) depending on the periods. When organic matter digestion levels were examined, it was seen that the highest OMD was in July with 58.71 %. Gas production was determined by incubations of 0, 3, 6, 12, 24, 48, 72, and 96 h, and the maximum gas measurement value was in September, July, and May, respectively. As a result of the study, STL can be a good alternative feed source for ruminants when adequate amounts of high-quality feed materials cannot be provided.

1. Introduction

When it comes to animal production, roughage and concentrate feeds are the first things that come to mind, especially in ruminant animal feeding. While concentrate feeds are given to provide the daily nutritional needs of animals, the roughage group is necessary for the animal to be efficient and healthy [1]. A significant aspect of evaluating grasslands is the quality of their forage nutrition, which

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influences livestock development and growth as well as the amount and quality of livestock products [2]. Roughages are plant-based feeds with high cellulose content but low protein and energy levels, used as fresh, dried, or silage feed for animals. They constitute the main part of ruminant diets [3]. Fiber, protein, and other nutrients are typically the most prevalent forage nutrition quality variables [4,5]. The digestibility of dry matter is the fundamental parameter that indicates the quality of forage resources when evaluating its quality. Increasing the protein level and lowering the cellulose concentration of forage can help improve its nutritional quality [6,7] This, along with the quantity of neutral, acidic detergent fibers and crude protein, provides the fundamental information to evaluate forage that is supplied to the cattle diet [8].

The roughage sources in our country are not sufficient to meet the requirements of our animals. Although the ratio of pasture-grazing areas to total land area is good compared to many countries, their yields are very low due to ecological conditions and misuse [9]. The woody group of pasture vegetation is "shrub and shrub-like trees," and the herbaceous group is composed of pasture plants [10]. Pasture plants host many different types and structures of plants. Pastures can host good quality forage plants that are beneficial for soil conservation and feed quality. Tree branches and leaves can form an important part of the diet for ruminant animals such as sheep, goats and deer [11]. Tree that can be used as an alternative feed source. Its leaves are classified as juicy roughage. Goat and animals, especially sheep, tree. They love to eat their leaves. Therefore agriculture fruit, ornamental and forest trees in enterprises. Its leaves are used for this purpose [12].

Styrax Tree (Styrax officinalis) is a shrub species from the woody plant group of the pasture vegetation, which grows mainly in Mexico, Central America, and the Mediterranean region [13]. The mountainous Styrax tree (ST) can reach up to 1.5–3 m in height. Its general distribution in Turkey ranges from sea level to 1000 m above sea level, and it can be found in many provinces of Western Anatolia and the Mediterranean region, usually in valley bottoms, open habitats, and northern slopes [14]. Styrax officinalis appears to be beautiful, being a deciduous small tree or a large shrub. The leaf is round-shaped and the leaf color is light green. The upper side of the leaf is smooth, the lower side is hairy. The length of the plants differs between two and 5 m. Its flowers are white, and the flower size ranges from 2 to 4 cm. 3–6 flowers combine to form a flower cluster. The petals of the flower form a short tube by joining at the bottom. Its fruit is cherry-sized and surrounded by a white cover. However known as the Tesbih tree in Turkey, it is also named "Deli Tesbih" in Ödemiş, "Havz" in Antakya, "Tesbih" in Adana and Muğla, "Mangıkı" in Tire, and "Domuz" in Bayındır [15].

Methane is a member of the greenhouse gases caused by anthropogenic activities that contribute most to global warming. Methane emissions released by ruminant animals into the atmosphere vary between 18 % and 33 %. The presence of metabolites (among others, coumarins, phenols, phenols and saponins) in a wide range of products in tropical trees may be alternatives to control rumen fermentation and relatively inactivate the rumen and limit enteric methane overall [16]. Determination of the amount of enteric CH_4 unraveling grazing systems is a challenge for all regions of the world, to increases especially depending on the quality of the pasture where the cattle are fed abundantly throughout the year. Ruminants are the ones involved in increasing CH_4 production in agriculture. mainly from the naturally occurring enteric CH_4 emission of the rumen process. feed fermentation [17,18]. Additionally, CH_4 levels emitted into the atmosphere also represent energy loss. For ruminant animals, it differs between 2 and 12 % depending on numerous factors, especially the type of feed [18,19]. Therefore, it is crucial to define emissions accurately.

This study was conducted to examine the nutritional content and methane production potential of the Styrax tree, a shrub species that grows in the Mediterranean region and serves as an valuable source of feed for small ruminants, particularly during the summer and autumn months. The goal was to highlight the potential feed value of the Styrax tree and to encourage its use in broader feeding systems. The study analyzed the nutritive values of the plant as well as its in vitro gas production, metabolic energy yield, and organic matter digestibility. The objective of this study is also to develop alternative feed sources regarding the potential of some trees, and their secondary metabolites to reduce methane emissions from ruminant animals.

2. Materials and methods

In the research, samples was collected from naturally grown STL in May, July, and September, which are three vegetation periods. STL were manually collected from 10 trees in each replication, with three replications for each vegetation period, and brought to the laboratory. The collected samples were first weighed for fresh weight, and then dried in the shade. The dry weights were determined after drying. The dried samples were sieved to pass through a 1 mm sieve and packaged for analysis of dry matter (DM), crude fat (CF), crude protein (CP), crude ash (CA), organic matter (OM), ADF, NDF, and in vitro gas measurements based on AOAC [20] and Van Soest et al. [21] methods.

The metabolic energy (ME) contents and in vitro OMD values of the STL used in the experiment were examined via the gas production technique (GPT) according to the method reported by Menke et al. [22]. To calculate gas production [22] in the study, the method was used described by Makkar et al. [23]. This technique depends on measuring the volume of gas generated when fodder is incubated with rumen fluid for a full day. An adequate volume of rumen fluid was obtained from yearling lambs slaughtered in slaughterhouses and added to a hot water bath that had already been set up, along with CO_2 to guarantee anaerobic conditions. An equal amount of rumen fluid was transferred into a thermos and filtered through four-layers cheesecloth, washing with CO_2. Rumen fluid and buffer solution were united at a ratio of 1:2 (V/V). 40 ml of buffered rumen fluid was conveyed into syringes containing STL samples (0.5 g) in quadruplicate. To obtain the blanks, 40 ml of buffered rumen fluid was transferred into four syringes without STL samples. For 24 h, every syringe was incubated in a water bath sustained at 39 °C. To calculate net gas production during the course of the 24-h incubation, gas and methane production were detected from syringes carrying STL sample. Net gas productions of STL samples were obtained after correction for blank and STL.

The methane concentration (%) of the gasses formed after 24 h of incubation was found using an infrared gas analyzer (Sensor Europe GmbH, Erkrath, Germany) [24] ME (MJ/kg DM) and OMD of styrax tree leaves were calculated via the equation of Menke &

Steingass [25]. Metabolic energy (ME) can be determined from equation (1) and organic matter digestibility (OMD) from equation (2) as follows:

$$\text{ME (MJ/kg DM)} = 1.68 + 0.1418\ \text{GP} + 0.073\text{CP} + 0.217\ \text{EE} - 0.028\text{CA} \tag{1}$$

$$\text{OMD (\%)} = 14.88 + 0.8893\ \text{GP} + 0.448\text{CP} + 0.651\text{CA} \tag{2}$$

where GP = 24 h of net gas production (mL 200 mg-1); CP = crude protein (%); EE = ether extract (%); and CA = ash content (%)

The methane content of the gas produced after 24-h incubation was found using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) [24] using the following equation (3).

Methane production (mL) = total gas production (mL) X percentage methane (%) (3).

To examine the differences between the means, one-way analysis of variance (ANOVA) was used with The SPSS 9.0 [26] package program. The Duncan comparison test was applied to determine the significance of the differences between the means.

3. Results and discussion

The raw nutrient contents of the forage used in the study are presented in Table 1. The effect of season on DM, CA, CP, CF, ADF, and NDF contents of the STL was statistically significant ($P < 0.01$). The DM, CA, CF, NDF, and ADF amounts were at their highest levels in September, while they were at their lowest levels in May. Compared to the May period, an increase of 9.8 % in ADF, 9.8 % in NDF, 23 % in CA, 30.5 % in CF, and 64 % in DM was observed in the September. The DM content of a feed represents the portion containing all the nutrients in that feed. The higher the DM content of a feed, the higher the likelihood that it is rich in nutrients [27].

The HP content of STL was statistically similar in May and July periods, and the lowest HP content was found in September period. The HP content in September period decreased by 17.5 % compared to May period.

According to the results of the analysis conducted on the STL, the DM content of the harvested plants in May was approximately 30 %, while it was around 40 % for those harvested in July and around 50 % for those harvested in September, reaching its highest level. There were increases in DM content parallel to maturity during the growing season. The results of the ash analysis of the STL collected according to the growing season showed that the ash content was approximately 8.58 % in May, 9.86 % in July, and around 10.56 % in September, reaching its highest level. As the DM content increased according to the growing season, the ash content also increased. The CP content of STL was approximately 18.81 % in May, 17.43 % in July, and around 15.52 % in September. As the maturity of the STL progressed, a decrease in CP content occurred. When studies conducted on tree and shrub leaves were examined, it was observed that our findings were in line with previous studies. Thus, plants with the highest CP content in the spring showed a decrease in crude protein content as growth progressed [12,28,29. [30]; [31]; [32]; [33]; [34]; [35]The change in nutrient content in plants is also related to physiological processes during growth and development. In the beginning of the growing season, plants show more active growth and have higher photosynthetic capacities. During this process, plants are more effective in synthesizing nitrogenous compounds required for high protein synthesis. Young cells have more protoplasmic substances with high protein level, while cell wall materials are lower [36,37]. There exists a positive correlation between the nutrition quality of herbage and CP [38,39].

The CF content in the shrub STL varies depending on the harvesting period, with the highest percentage of around 4.4 % occurring in July and a similar rate of 4.3 % in September. The lowest CF content was detected during the May harvest period. According to the analysis conducted on STL, the NDF content was approximately 37.5 % in the plants harvested in May, 39.0 % in those harvested in July, and around 41.5 % in those harvested in September, reaching the highest level during this period. There were increases in NDF level parallel to maturity during the vegetation period. The NDF value in the leaves was significantly affected by seasonal changes. The composition of tree leaves varies due to translocation, which occurs seasonally. As mentioned earlier, translocation during the aging period changes the leaves' elements, particularly protein content. The seasonal change in the ratio of the cell wall components of STL is thought to be related to translocation. The NDF content is consistent with the study conducted by Kamalak et al. [40]. Similarly, in other studies, it was revealed that shrub and tree leaves have less fibrous compounds, more young cells, and intracellular components compared to stems or thin shoots [41–43]. In addition, the NDF content was lower in the spring season compared to the other two periods, and there were increases in NDF ratios with the progress of the maturity period.

Nutrient compositions of shrubs may vary depending on the season or even the month [44]. With the start of growth in spring, the formation of new shoots and leaves rich in protein content accelerates in the bushes. In the summer months, the protein content decreases and the amount of cell components increases. The amount of cell components grows and the protein content falls throughout the summer [45,46]. Understanding what kinds of grazing animals like in bush pastures is essential to the sustainability of grazing animals (Ammar et al., 2005- [47]. The proportion of shrubs that animals eat on a daily basis varies according on the kind, variety,

Table 1
Nutrient content (%) of Styrax Tree leaves in different growth stages.

Periods	DM	CA	CP	CF	NDF	ADF
May	29.72 ± 0.91^a	8.58 ± 0.18^a	18.81 ± 0.62^a	3.21 ± 0.08^a	37.78 ± 0.36^a	27.28 ± 0.26^a
July	42.07 ± 1.36^b	9.86 ± 0.17^b	17.43 ± 0.29^a	4.30 ± 0.16^b	39.09 ± 0.37^b	28.21 ± 0.26^b
September	48.74 ± 0.57^c	10.56 ± 0.20^c	15.52 ± 0.13^b	4.19 ± 0.11^b	41.50 ± 0.34^c	29.95 ± 0.24^c
P	**	**	**	**	**	**

^{abc}: *The difference between the groups in the same column is statistically significant. **P < 0.01.*

stage of vegetation, and environmental factors [48]. According to Perevolotsky et al. [49], goats on bush pastures can form more than 60 % of the feed they consume annually. Darcan et al. [50]. States that goat farming is the only source of life for rural people in the mountainous regions of the Eastern Mediterranean and that they have no other alternatives due to topographical reasons.

According to the analysis results, there have been increases in ADF level depending on the periods, with values around 27 % (May), 28.5 % (July), and 30 % (September). As the harvest time progresses, ADF content has also increased in parallel.

In many studies conducted in various ecological conditions, it has been reported that the CP content is high and the ADF content is low in the early development stages of shrub and tree species, while these values are reversed with maturity [30,45–50]. The climate and soil conditions in which plants grow also affect the change rate of cell wall components. For example, plants grown in high rainfall areas may have higher ADF content, while those grown in dry and hot areas may have lower ADF content. Therefore, the geographical region, climate, and soil conditions in which plants like the tespih bush grow can have a significant impact on the CP and ADF content of the plants. For these reasons, it is thought that leaf samples collected from STL in the spring season may have higher raw protein and lower ADF content.

Moreover, since plants usually complete their growth and maturation in autumn, they may have lower dry matter content and higher non-structural intracellular components at the beginning of the growth period compared to advanced growth stages [51]. In other words, fibrous compounds are generally more abundant in cell walls, while components of the cell wall are more prevalent in older tissues than in young tissues [41].

The ME levels of the STL varied significantly among the different periods ($P < 0.05$) as shown in Table 2. The May and September periods were in the same group in terms of ME, and were lower than the July period. The metabolic energy content ranged between 8.86 and 10.06 (MJ/kg DM) depending on the period. The findings on ME content obtained from the study are consistent with the values reported by Tatlıyer et al. [12], Karabulut et al. [52] and Kamalak et al. [11].

Statistical analysis showed that OMD levels were significant ($P < 0.01$) in the studied STL. The highest OMD level was obtained in July with a percentage of 58.71 %.

The observed differences in the ME and OMD levels may be due to the genetic structure of the species as well as their growth stages and plant parts. This is because plant leaves have lower amounts of fibrous compounds compared to thin shoots and stems [51,53]. Therefore, leaf samples with lower NDF and ADF content can be more digestible and have higher metabolic energy content. Herbage digestibility and acid detergent fiber (ADF) have a negative correlation [39,54].

In other words, it has been noted that fibrous compounds are typically found in cell walls and are more abundant in older tissues than in young tissues [41]. As a result, in advanced growth stages of plants, leaves and young shoots become lignified, cell protoplasm compounds decrease, and consequently, lower digestibility and ME content emerge [12,37,43,55]. For these reasons, in the beginning of the growth period, which is in the spring season, leaves may have higher digestibility and ME content, and as the maturity period progresses, they may have lower values. Similar results have been reported by various researchers, and it has been stated that DMD, SE, and ME contents are higher in the leaves of plants and early growth stages [35,56–59]. In instance, as the plant grew more mature, its cell wall (NDF and ADF) content declined [60].

The gas production amounts of STL for different periods increase parallel to the incubation time (Table 3). However there are differences in gas production amounts for all periods according to the incubation time, it generally occurs at the highest level in September and at the lowest level in May (Fig. 1).

During the incubation period, gas measurements were recorded at 3, 6, 12, 24, 48, 72, and 96 h, and the gas production values were lowest at 3 h but highest at 96 h.

An increase in ADF levels in the structure of feed of the STL reduces the digestibility rate of the feed, while an increase in NDF levels reduces feed intake, causing the animal to feel full and limiting their feed intake and suitability. Furthermore, Herbage palatability has a negative correlation with NDF [5,54]. On account of the negative effect of high ADF and NDF on feed digestibility and intake, feeds with ideal levels of these values are preferred when used in rations [61–63]. Populations are mostly classified as first quality based on ADF and NDF contents, while only a small proportion is classified as second quality for roughage [62].

The variations in plant stem to leaf ratios in plants and the occurring differences in CP, NDF, and ADF levels lead to variations in the amount of gas produced by populations. Aslan [64] found a negative relationship between NDF and ADF content in roughages and their digestibility. ADF and NDF contents resulted in low organic matter digestibility (OMSD) in wheat straw, a rich roughage. It was determined that there was a negative relationship between the CA content of feeds and the total gas they produced. The results of this research are consistent with the results of other studies conducted by researchers such as Abdulrazak et al. [65] and Kamalak [66], Tatlıyer et al. [12], and these results support our study.

Table 2
Levels of ME (MJ/kg DM) and OMD (%) in Styrax tree leaves at different periods.

Periods	ME (MJ/kg DM)	OMD (%)
May	8.86^{a}	54.62^{a}
July	10.06^{b}	58.71^{b}
September	9.12^{a}	54.55^{a}
SEM±	0.25	1.22
P	**	*

^{ab}: The difference between the groups in the same column is statistically significant. *$P < 0.05$ **$P < 0.01$.

Table 3
Gas production amounts (ml/200 mg DM) of Styrax tree leaves in different periods.

Incubation Time (hours)	May	July	September
3	8.60 ± 1.42	13.20 ± 0.75	14.54 ± 1.28
6	14.29 ± 2.79	22.26 ± 1.88	23.80 ± 1.88
9	16.18 ± 3.45	25.23 ± 1.41	25.97 ± 2.27
12	24.50 ± 3.47	32.80 ± 4.52	34.49 ± 4.5
24	34.56 ± 3.30	39.76 ± 2.37	35.99 ± 2.66
48	43.63 ± 5.28	43.38 ± 1.53	45.23 ± 2.32
72	51.88 ± 2.04	51.04 ± 3.50	50.98 ± 3.26
96	52.67 ± 4.68	56.73 ± 2.44	58.66 ± 3.87

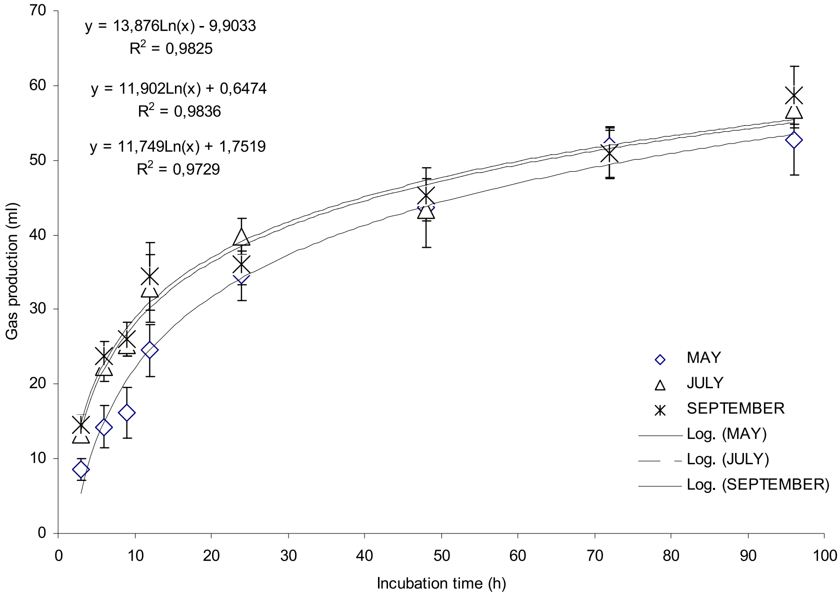

Fig. 1. Gas production amounts (ml/200 mg DM) of Styrax tree leaves in different periods.

4. Conclusion

This study revealed differences in nutrient content of STL according to vegetation periods, which is an important feed source for ruminants in many regions of Turkey, and concluded that it can be used as a good alternative feed source that can replace roughage. In addition, this study predicts that animals grazing on STL, which are an indispensable feed source for Mediterranean climate zone pastures, especially during dry summer periods, can meet their nutrient requirements. Therefore, STL can be a good alternative feed source for ruminants when adequate amounts of high-quality feed materials cannot be provided.

CRediT authorship contribution statement

İlyas Balyen: Writing – review & editing, Investigation, Data curation. **Ayfer BOZKURT KİRAZ:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ayfer Bozkurt Kiraz reports financial support was provided by Harran University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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