In Situ and *in Vitro* Nutritive Value Assessment of *Styrax Officinalis* L. as an Alternative Forage Source for Goat Feeding

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ABSTRACT

The current study aimed to determine *Styrax officinalis* L. (SO) leaf's nutritive value, collected at four phenological stages, pre-flowering (PF), flowering (FL), seed linkage (SL), and fruiting (FR) by *in situ* and *in vitro* experiments. The ruminal degradability of dry matter (DM) and crude protein (CP) and *in vitro* gas production (GP) of SO leaves were measured using three rumen fistulated mature Saanen goats. Significant differences between chemical compositions of the SO leaves collected at different phenological stages were observed (P<0.001). The DM, CP, ether extract (EE), and ash values of SO leaves ranged between 29.16 to 45.63%, 10.11 to 19.79%, 3.40 to 5.85%, and 4.71 to 6.49% during the different phenological stages (PF, FL, SL and FR, respectively). Cell wall components of SO leaves showed a cubic trend due to their capability to form new shoots after grazing. The effective DM and CP degradability of SO leaves ranged between 66.91 to 77.93% and 64.92 to 84.57%, which means an average value for animals fed at approximately maintenance level when rumen outflow rate (r) is equal to 0.02 h^{-1}. Significant differences between the SO leaves collected at different phenological stages were observed in GP at all incubation times (P<0.05 and P<0.001). After 96 h incubation, the gas produced ranged between 20.68 to 27.53 mL/200 mg DM of the substrate. The research findings clearly indicate that degradability of DM, CP, and ME content of SO leaves ranged between moderate to high and significantly affected by phenological stages, however, they could be utilized until the end of the FL stage as forage sources.

Keywords: *Styrax officinalis* L. leaves, Ruminal degradability, Digestibility, Gas production, Chemical composition

1. Introduction

Goats, well-adapted species to marginal areas, can benefit from shrubs effectively, while other species cannot utilize them. In Turkey, goat breeding is characterized by low input usage and is carried out depending on natural conditions. Thus, natural pastures have an essential role in goat feeding. Therefore, shrublands in the Mediterranean climate zone represent valuable feed sources, especially in goats (Perevolotsky et al. 1998). In general, the quality of shrubland depends on the type, distribution, and nutrient composition of the plant. Indeed, shrublands become more important in meeting these animals' nutrient demands in dry seasons (Tölü 2009).

The nutrient composition of shrubs may vary according to seasons or even months (Rogosic et al. 2006). While new shoots and leaves rich in protein content accelerate in spring, protein content decreases, and cell wall components increase in summer. Acknowledgement of the changes in the shrubs' nutrient content throughout the year makes it an important source to decide the grazing season, determine additional feed strategies, establish the pasture-animal relationship correctly, and increase the economic and biological efficiency of breeding activities (Metera et al. 2010). In Turkey, shrublands are widely used for goat feeding throughout the year in some areas. Therefore, determining the animals' grazing preferences in shrublands is critical for these areas' sustainability. While there is a limited number of studies that were performed in Turkey aimed to determine the annual feed production of the shrubs, the change of feeding potential, and the grazing capacity, there are many studies on the nutritional composition of shrublands and consumption preferences of different species in these areas and about determining the feeding potential, *in vitro* gas production, and *in sacco* degradability characteristics of some of them in the literature (Dziba et al. 2003; Boubaker et al. 2004; Kamalak et al. 2004; Ammar et al. 2005; Moujahed et al. 2005; Karabulut et al. 2006; Rogosic et al. 2006; Salem et al. 2006; Bruno-Soares et al. 2011; González-Pech et al. 2021).

Styrax officinalis L. (SO), which belongs to the *Styracaceae* family, is a deciduous plant in a dwarf tree or bush and is widely distributed in America, Mexico Mediterranean countries, e.g., Turkey, France, and Greece (Jaradat 2020). Although SO has deep roots, it has developed a root system that allows to benefit more from surface water due to their adaptation to drought (Mahall et al. 2010). The average 180-day life cycle starts with buds' blooming in early spring and ends with senescence in August-September. The main factor determining the growth and the number of leaves and flowers on the plant is the amount of rainfall in the previous year and varies according to annual rainfall (Mahall et al. 2010). The Phoenicians, Egyptians, and Romans used the resins of this plant for incense and treatment. It is widely found in the south, west, and northern parts of Turkey. Besides, the *Styracacea* family, e.g., *S. japonicum*, *S. formosanus*, *S. obassia*, *S. macranthus*, and *S. officinalis*, is known for its various biological activities in the form of insecticidal, fungicidal, antimicrobial, antidiabetic, antiproliferative, and cytotoxic effects (Łyczko et al. 2020; Yesilyurt & Cesur 2020).

Briefly, it is possible to meet some of the nutrient requirements of goats from plant leaves and fruits that grow in shrublands in dry seasons. One of them is SO, which is widely distributed in the maquis vegetation. Therefore, the current study aimed to determine the nutritive value of SO leaf, collected at four phenological stages, by *in situ* and *in vitro* experiments.

2. Material and Methods

The present study was carried out from April to August 2019 in Balikesir, Turkey. The animal care and handling procedures were reviewed and approved by the Sheep Breeding Research Institute's Ethical Committee (Approval number: 3451976).

2.1. Forage samples

The leaf samples of SO was collected by hand-clipping from at least 20-30 different individual SO shrubs at pre-flowering (PF), flowering (FL), seed linkage (SL) and fruiting (FR) stages in four different district of Balikesir, Turkey (Bandirma (B), Erdek (E), Gönen (G), Manyas (M)) via three different sampling point. The amount of dry matter (DM) in the fresh form, collected from each sampling point, was determined on the same day by drying approximately 10 g of fresh material in an oven at 102 °C for at least 16 hours until it reaches a constant weight. Fresh leaves were dried in air-conditioned rooms (24 ± 2 °C) by inverted several times a day for a week and were ground to pass through a 3 mm, 1 mm, and 0.5 mm sieve kept in the refrigerator until analysis.

2.2. Chemical analysis

The proximate analysis of SO leaves used in the *in situ* and *in vitro* studies was performed according to Weende's analysis by using AOAC (1990) methods. Briefly, dry matter (DM) of SO was determined by drying the SO leaves at 102 °C overnight, and ash content was determined by ashing the SO leaves in a muffle furnace at 550 °C for 3 h. The nitrogen (N) content of SO samples was measured by the Kjeldahl method and multiplied by 6.25 to get the crude protein (CP) ratio. To analyze the Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of SO, a method was used described by Van Soest et al. (1991). The condensed tannin (CT) content of SO samples were determined by Sun et al. (1998). While the samples milled through 1 mm were used for the proximate analysis with cell wall contents, milled through 0.5 mm was used to determine SO leaves' CT contents.

2.3. In situ DM and CP degradation

Three adult male Saanen goats, about 3 years old ages and 74.27 ± 1.03 kg live weight (Mean ± SE), fitted with a permanent rumen cannula were used to measure the rate and extent of digestive DM and CP loss (digestibility, effective degradability) of SO leaves by using the in situ nylon bag technique (Ørskov & McDonald 1979). Goats received a good quality alfalfa hay and concentrate (60:40) at about 1.25 times greater than maintenance level twice a day, at 09:00 and 16:30 at an equal amount. Nylon bags purchased from Ankom Technology (R510 forage bag, 5 x 10 cm size, 50 ± 10 μ porosity; ANKOM Technology, Macedon NY, USA). Approximately 3 g of SO leave, milled through 3 mm, were placed in each nylon bag and incubated in the rumen of each of the three goats for specified periods (3, 6, 12, 24, 48, and 72 h). Four bags, each containing one of the SO leaves collected from different phenological stages, were placed in each goat's rumen and then withdrawn at once after remaining in the rumen for the specified incubation time. Upon removal from the rumen, bags were immersed in a bucket of cold water, transferred into the laboratory, washed in running cold tap water for 2 minutes, and then washed in the cold rinse cycle (15 min) of a washing machine. Zero time disappearance was obtained by similarly washing un-incubated bags. The washed bags were dried in an air-forced oven (60 ± 2 °C, 48 h) and weighed to determine DM disappearance. Digestion characteristics of DM and CP of SO leaves were calculated using equations 1 and 2 (Ørskov & McDonald 1979):

$$Y = a + b(1 - e^{-ct}) \tag{1}$$

$$P = a + \frac{(b \times c)}{(c+r)} \tag{2}$$

Where: Y, is the disappearance rate of DM or CP of SO leaves at time t (%); a, an intercept representing the portion of DM or CP of solubilized SO leaves at zero time incubation (%); b, the portion of DM or CP of SO leaves that is readily degraded in the rumen (%); c, the rate constant of fraction b (% h^{-1}); t, is the time of incubation (h); P, is the effective degradability of DM or CP of SO leaves (%); r, is the rate of outflow from the rumen (% h^{-1}; an outflow rate of 0.02, 0.05, and 0.08 was applied).

2.4. In vitro gas production

In vitro incubations of SO leaves were carried out using the ANKOM RF Gas Production System (ANKOM Technology, Macedon NY, USA). Rumen fluid was obtained from three adult male Saanen goats before morning feeding. On each occasion, approximately 200 mg SO leaves sample (dry weight), milled through 1 mm, were incubated in triplicate in 250 mL glass bottles. The glass bottles were pre-warmed at 39 °C before the injection of 100 mL rumen fluid-buffer mixture (without trypticase; 20/80, v/v) by following the procedure of Goering & Van Soest (1970). All the glass bottles containing rumen fluid-buffer mixture and SO leaves samples were incubated for specified times (0, 3, 6, 12, 24, 48, 72, and 96 h). Total gas volume was corrected for blank incubation. Cumulative GP data were fitted to Ørskov & McDonald (1979) model using the NEWAY computer package program according to equation 3.

$$Y = a + b(1 - e^{-ct}) \tag{3}$$

Where: Y, is the gas production volume (GP) of SO leaves at time t (mL /200 mg DM); a, gas production volume from immediately soluble fraction of SO leaves (mL); b, gas production from the insoluble fraction of SO leaves (mL); c, gas production rate constant for the insoluble fraction of SO leaves (mL/h); t, the time of incubation.

The metabolizable energy (ME, MJ/kg DM) and organic matter digestibility (OMD, % DM) contents of SO leaves were calculated using equations of Menke et al. (1979) (Equations 4 and 5).

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP \tag{4}$$

$$OMD\ (\%\ DM) = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ Ash \tag{5}$$

Where: GP, is 24 h net gas production (mL/200mg); CP, crude protein.

2.5. Statistical analysis

In situ DM and CP degradation, data of chemical components, and *in vitro* GP and kinetics were analyzed applying a randomized complete block design using SAS (2016) pocket program. The least-squares means were compared using Tukey's multiple comparison tests. The model used for the least-squares (LS) analysis was as follows:

$$Y_{ijk} = \mu + B_i + S_j + e_{ijk} \tag{6}$$

Where: Y_{ijk}, is the variable studied; B_i, is the block; (B, E, G, M), S_j, is the phenological stages (PF, FL, SL, and FR); e_{ijk}, is the residual error. Linear, quadratic, and cubic trends of the phenological stage were analyzed according to orthogonal polynomial contrasts.

3. Results and Discussion

The chemical composition of the SO leaves collected at four different phenological stages is presented in Figure 1. Significant differences were observed between chemical compositions of the SO leaves collected at different phenological stages (P< 0.001). DM contents of SO leaves ranged between 29.19 to 45.63% and increased with maturity, except the FL stage. In contrast to DM, SO leaves' CP content decreased with increased maturity and ranged between 10.11 to 19.79%. CP content of SO leaves collected at the PF stage was significantly higher than those gathered at the other three stages (FL, SL, and FR). While the highest CP (19.79%) and ash (6.49%) contents were observed at the PF stage; the highest DM (45.63%), EE (5.85%), ADL (10.14%), CT (22.75%) contents were observed at FR stage as expected with increased maturity. CT content of SO leaves ranged between 6.42 to 22.75%. Previous researchers have demonstrated that with the plant's increased maturity, the DM ratio increased while the CP ratio decreased (Mountousis et al. 2008; Tolunay et al. 2009; Ataşoğlu et al. 2010). The results obtained in the DM and CP ratio in the study are consistent with the literature reports on this subject.
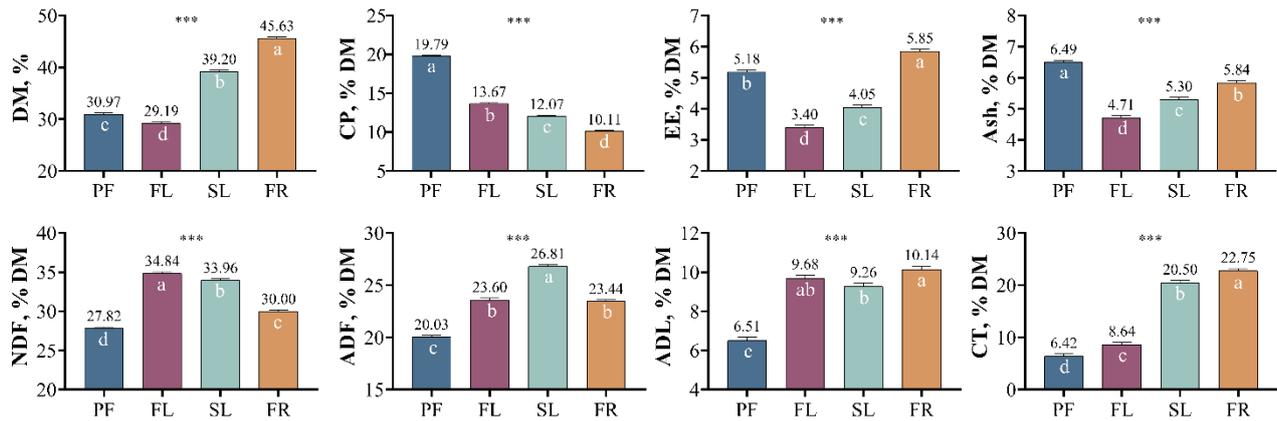
Figure 1- Chemical composition of *Styrax officinalis* L. leaves collected at different phenological stages

PF: Pre-flowering stage, FL: Flowering stage; SL: Seed linkage stage, FR: Fruiting stage; DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CA: Crude Ash; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; CT: Condensed Tannin
The values with different letters (a, b, c, d) in each graph is statistically different (P<0.05);
***: P<0.001.

In contrast, the increased level of DM of SO leaves with the SL stages in parallel to weather conditions, NDF, and ADF values were found to be decreased. These results may be accepted as an indicator that SO's leaf formation continues from new shoots after grazing. It was well documented in the literature that lignification was increased in plants as the maturity was increased (Parissi et al. 2005). In the present study, the ADL content of SO leaves was increased as the maturity was increased.

In a study conducted to determine the anti-methanogenic properties of some tree leaves containing tannins by in vitro gas production test, the DM, CP, EE, Ash, NDF, and ADF content of SO leaves was found to be 34.26%, 11.64%, 9.28%, 13.33%, 28.95% and 22.94% respectively (Şimşek & Kamalak 2019). In another study conducted to determine the yield and nutrient composition of shrubs in the pastures in Anti-Taurus Mountain, the CP, ADF, and NDF content of SO leaves was determined as 10.60%, 23.9%, 40.2%, in Şavşak and 11.6%, 27.2%, 43.1% in Çandır, respectively (Kökten et al. 2010). However, it was not specified in which vegetation period or phenological stage the samples were taken in both studies. For this reason, it would not be easy to make an adequate comparison between the data obtained in our research and the literature reports. On the other hand, the results obtained in our study were found to be higher than the reported values of the SO leaves collected by Ertekin et al. (2019) at the FR stage.

The profiles for DM and CP disappearance of each phenological stage are illustrated in Figure 2, and degradability parameters are given in Tables 1 and 2. The mean rates of DM and CP disappearance were greater for the PF stage compared to the other three phenological stages (Figure 2). After a 48 h incubation period, DM disappearances between PF - FL and SL - FR were close to each other (Table 1). Except for the 12 h incubation period (P<0.01), all the incubation periods, degradability parameters, and effective degradability of SO leaves were significantly affected by the phenological stages (P<0.001). Also, significant differences between the phenological stages in terms of effective degradability of DM obtained from different rumen outflow rates were observed. While the SO leaves' highest effective degradability was observed at PF stages, the lowest effective degradability of SO leaves was marked at SL stages. Like the disappearances of DM of SO leaves, the CP degradability was significantly affected by the phenological stages (P<0.01 and P<0.001) (Table 2). There was a significant decrease in CP degradation in the rumen with increased maturity of SO. Approximately 27.94% decrease was observed between the beginning (PF) and the end of the vegetation period (FR) for the readily degradable part of CP of SO leaves.
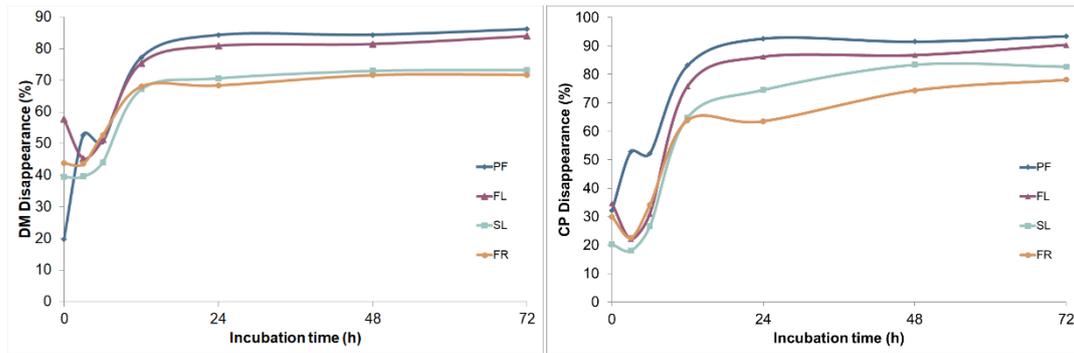
Figure 2- Degradation curve of dry matter and crude protein of *Styrax officinalis* L.

PF: Pre-flowering stage; FL: Flowering stage; SL: Seed linkage stage; FR: Fruiting stage; DM: Dry Matter; CP: Crude Protein

Table 1- Degradability coefficients and effective degradability of DM of *Styrax officinalis* L.

	PF	FL	SL	FR	SEM	M	PS Effect L	Q	C
0	19.87^z	57.71^x	39.43^y	43.84^w	0.161	***	NS	NS	***
3	52.73^x	45.28^w	39.62^y	43.61^{wy}	0.883	***	*	***	***
6	50.51^x	51.19^x	44.03^w	52.68^x	0.699	***	NS	NS	***
12	77.15^x	75.34^{xw}	67.11^y	68.03^{wy}	1.664	**	**	**	**
24	84.29^x	80.89^w	70.65^y	68.38^z	0.233	***	***	***	***
48	84.34^x	81.45^x	73.01^w	71.63^w	0.691	***	***	***	***
72	86.13^x	83.93^x	73.23^w	71.74^w	0.826	***	***	***	***
c	0.146^x	0.062^y	0.085^w	0.099^w	0.004	***	NS	NS	NS
a	22.05^z	48.35^x	34.90^y	40.57^w	0.489	***	NS	*	NS
b	63.57^x	36.92^w	39.67^w	31.70^y	0.833	***	NS	NS	NS
ED[1]	77.93^x	76.28^x	66.95^w	66.91^w	0.374	***	***	***	***
ED[2]	69.36^x	68.81^x	59.79^w	61.58^w	0.303	***	**	*	***
ED[3]	63.07^w	64.49^x	55.25^z	58.05^y	0.281	***	*	NS	***

IT: Incubation time (h); PS: Phenological stages; PF: Pre-flowering stage; FL: Flowering stage; SL: Seed linkage stage; FR: Fruiting stage; SEM: Standard error of means; M: Main effect; L: Linear effect; Q: Quadratic effect; C: Cubic effect; a: the fraction of readily degradable DM (%); b: the fraction of degradable DM at a measurable rate (%); c: degradation rate of DM (% h^{-1}); ED[1]: effective degradability of DM (%) with a rumen outflow rate of 0.02; ED[2]: effective degradability of DM (%) with a rumen outflow rate of 0.05; ED[3]: effective degradability of DM (%) with a rumen outflow rate of 0.08; The values with different letters (x, w, y, z) in the same row are statistically different (P<0.05), NS: Not Significant; *: P<0.05, **: P<0.01, ***: P<0.001.

Table 2- Degradability coefficients and effective protein degradability of *Styrax officinalis* L.

IT	PF	FL	SL	FR	SEM	M	PS Effect L	Q	C
0	32.18^w	34.61^x	20.28^y	29.96^w	0.492	***	***	NS	**
3	52.85^x	22.14^w	18.03^w	22.59^w	2.017	***	**	***	***
6	52.16^x	30.97^{wy}	26.57^y	34.13^w	1.646	***	*	***	***
12	83.16^x	75.85^{xw}	64.86^{wy}	63.86^y	2.501	**	***	***	**
24	92.58^x	86.14^x	74.55^w	63.53^y	1.861	***	***	***	***
48	91.48^x	86.69^x	83.39^x	74.36^w	1.816	**	***	***	***
72	93.43^x	90.31^x	82.71^w	78.12^y	0.931	***	*	*	***
c	0.110^x	0.072^w	0.072^w	0.063^w	0.005	**	NS	NS	NS
a	31.50^x	21.13^w	11.60^y	22.70^w	0.772	***	NS	***	***
b	62.72^w	71.45^x	74.15^x	56.00^y	1.339	***	NS	**	**
EPD[1]	84.57^x	76.97^w	69.53^y	64.92^z	0.437	***	***	***	***
EPD[2]	74.63^x	63.20^w	55.24^y	53.63^y	0.553	***	***	***	***
EPD[3]	67.83^x	54.88^w	46.61^y	47.14^y	0.588	***	***	***	***
BP[1]	15.43^z	23.03^y	30.47^w	35.08^x	0.437	***	***	***	***
BP[2]	25.37^y	36.80^w	44.76^x	46.37^x	0.553	***	***	***	***
BP[3]	32.17^y	45.12^w	53.39^x	52.86^x	0.588	***	***	***	***

IT: Incubation time (h); PS: Phenological stages; PF: Pre-flowering stage; FL: Flowering stage; SL: Seed linkage stage; FR: Fruiting stage; SEM: Standard error of means; M: Main effect; L: Linear effect; Q: Quadratic effect; C: Cubic effect; a: the fraction of readily degradable CP (%), b: the fraction of degradable CP at a measurable rate (%); c: degradation rate of CP (% h^{-1}); EPD[1]: effective degradability of CP (%) with a rumen outflow rate of 0.02; EPD[2]: effective degradability of CP (%) with a rumen outflow rate of 0.05; EPD[3]: effective degradability of CP (%) with a rumen outflow rate of 0.08; BP[1]: bypass protein (%) with a rumen outflow rate of 0.02; BP[2]: bypass protein (%) with a rumen outflow rate of 0.05; BP[3]: bypass protein (%) with a rumen outflow rate of 0.08. The values with different letters (x, w, y, z) in the same row are statistically different (P<0.05); NS: Not Significant;*: P<0.05; **: P< 0.01; ***: P< 0.001

The rapidly degradable (a) DM and CP fraction of SO leaves are presented in Tables 1 and 2. While the PF stage (22.05%) had a lower readily degradable DM, the FL stage (48.35%) had higher readily degradable DM. On the other hand, the lowest readily degradable CP fraction was observed at the SL stage (11.60%), while the highest one was the PF stage (31.50%). As shown in Tables 1 and 2, the effective degradability DM and CP of SO leaves, especially at heavily grazed at PF and FL stages, were moderate to high. To our knowledge, there is no information in the literature about *in situ* digestibility of SO leaves. However, compared to the *in situ* studies done with some trees and shrubs in the literature, SO leaves' digestibility, collected during the heavily grazed, for 48 h was higher than some exotic trees and shrubs *Spondias mombin* L., *Antiaris toxicaria* Lesch., *Baphia nitida* Lodd., *Grewia carpinifolia* Juss., *Griffonia simplicifolia* (DC.) Baill., *Calliandra calothyrsus* Meisn., *Acacia brevispica* Harms, *Acacia tortilis* (Forssk.) Hayne, *Acacia seyal* Delile, *Acacia nilotica* (L.) Delile, *Acacia mellifera* (M.Vahl) Benth; was similar to *Thespesia populnea* (L.) Sol. ex Corrêa, *Ficus exasperata* Vahl.; was lower than *Acacia nubica* Benth (Kaitho et al. 1993; Apori et al. 1998; Abdulrazak et al. 2000). The degradability of CP of SO leaves was higher than *S. Mombin*, *B. nitida*, *G. Carpinifolia*, *G. Simplicifolea*, *G. Oppositifolia*, *Ziziphus mauritiana* Lam.; was similar to *A. Toxicaria*, *F. Exasperata;* was lower than *T. Populnea* (Apori et al. 1998; Khan et al. 2009).

The GP, estimated parameters of GP, ME, and OMD of SO leaves collected at different phenological stages are given in Table 3. The GP of SO leaves increased with increasing time of incubation. After 96 h incubation, the gas produced ranged between 20.68 to 27.53 mL/200 mg DM of the substrate. Significant differences between the SO leaves collected at different phenological stages in terms of GP at all incubation times were observed (P<0.05 for 48, 72, and 96 h; P<0.001 for 3, 6, 12, and 24 h). In contrast to incubation time, no significant differences were observed for the estimated GP parameters (P>0.05).

Table 3- *In vitro* gas production kinetics, Metabolisable Energy and Organic Matter Digestibility of *Styrax officinalis* L.

IT	PF	FL	SL	FR	SEM	M	PS Effect		
							L	Q	C
3	12.24^x	8.53^w	8.52^w	9.20^w	0.31	***	***	***	***
6	16.39^x	12.51^w	12.19^w	12.95^w	0.39	***	***	***	***
12	19.36^x	15.78^w	15.39^w	16.65^w	0.48	***	**	***	***
24	21.25^x	18.41^w	17.61^w	19.08^{xw}	0.59	***	*	**	**
48	23.87^x	20.49^{xw}	18.12^w	20.78^{xw}	1.21	*	NS	*	NS
72	25.59^x	22.32^{xw}	19.52^w	21.45^{xw}	1.44	*	NS	NS	NS
96	27.53^x	23.97^{xw}	20.68^w	22.69^{xw}	1.67	*	NS	NS	NS
a	1.05	1.23	0.38	1.02	0.23	NS	NS	NS	NS
b	23.55	20.82	19.34	20.86	1.31	NS	NS	NS	NS
a + b	24.60	22.05	19.71	21.88	1.47	NS	NS	NS	NS
c	0.174	0.131	0.185	0.175	0.018	NS	NS	NS	NS
Gas	21.25^x	18.41^y	17.61^y	19.08^{xy}	0.59	***	*	**	**
ME	6.26^x	5.49^y	5.30^y	5.36^y	0.08	***	***	***	***
OMD	43.46^x	37.78^y	36.36^y	36.65^y	0.52	***	***	***	***

IT: Incubation time (h); PS: Phenological stages; PF: Pre-flowering stage; FL: Flowering stage; SL: Seed linkage stage; FR: Fruiting stage; SEM: Standard error of means; M: Main effect; L: Linear effect; Q: Quadratic effect; C: Cubic effect; a: the gas production from the immediately soluble fraction (mL); b: the gas production from the insoluble fraction (mL); c: the gas production rate constant for the insoluble fraction (b); a + b: potential gas production (mL); Gas: Gas volume after 24 h of incubation; ME: Metabolizable Energy (MJ/kg DM); OMD: Organic Matter Digestibility (% DM). The values with different letters (x, y) in the same row are statistically different (P<0.05); NS: Not Significant;*: P<0.05; **: P< 0.01; ***: P<0.001

At all incubation times, the GP of SO leaves collected at the PF stage were significantly higher than those gathered from the other three stages. The relationship between obtained GP and phenological stages from this study showed a cubic trend extending the grazing period. These results were not in agreement with such studies, which showed a decreasing trend as the plant's period increased (Sileshi et al. 1996; Lee et al. 2000; Kamalak 2006). On the other hand, the GP of SO leaves were relatively lower than other shrubs such as *Atriplex amnicola*, *Atriplex nummularia*, *Atriplex semibaccata Acacia saligna*, *Maireana brevifolia, Rhagodia preissii* (Norman et al. 2010). The lower GP may be related to the species of rumen fluid taken and the high propionate production levels of SO leaves during fermentation. These statements are in agreement with Van Soest (1994) and Getachew et al. (1998).

Based on the estimated parameters of GP, the readily soluble (a), insoluble (b), and gas production rate (c) were not significantly affected by the phenological stages (P>0.05). This result may be related to the large variety of chemical composition cell wall structure of SO leaves. Givens et al. (2000) stated that the feed's chemical composition and cell wall structure are often influenced the degradation parameters. Ndlovu & Nherera (1997) noted that approximately 25% of the gas production variation was related to changes in the feed's ADF and NDF content. The findings of this research are in agreement with the results of those researchers.

4. Conclusions

The present study was designed to better understand the importance of SO leaves for goat feeding in shrublands by using *in situ* and *in vitro* methods. Although the findings should be interpreted with caution, this study has several strengths. Firstly, the findings clearly indicate that degradability of DM, CP, and ME content of SO leaves were ranged between moderate to high and significantly affected by phenological stages. Secondly, the nutritional composition of SO leaves depicted them as good forage sources for goats until the end of the FL stage. Thirdly, SO has the ability to give new shoots in parts that are broken, cut, or grazed for any reason, allowing the grazing period to extend a little more. On the other hand, there is a limitation need to be noted regarding the grazing or feeding with SO leaves, which lies in the fact that the high CT levels. Further *in vivo* research is required to better understand voluntary consumption of SO leaves by goats and reduce the limiting effect of high CT levels on feed intake.

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