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Feed value of dried and ensiled paulownia (*Paulownia* spp.) leaves and their relationship to rumen fermentation, *in vitro* digestibility, and gas production characteristics

Hülya Özelçam[1], Hasan Hüseyin İpçak[2]*, Sema Özüretmen[1], Önder Canbolat[3]

[1] Ege University, Faculty of Agriculture, Department of Animal Science, İzmir, Turkey.
[2] Dicle University, Faculty of Agriculture, Department of Animal Science, Diyarbakır, Turkey.
[3] Bursa Uludağ University, Faculty of Agriculture, Department of Animal Science, Bursa, Turkey.

ABSTRACT - The study aimed to evaluate the potential use of dried or ensiled paulownia (*Paulownia* spp.) leaves as roughage source for ruminants. Paulownia tree leaves were collected from one-year-old hybrid (C-125, CAR, and TF-33 clones) trees. Dried paulownia leaves of the clones were different in dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NfE) and similar in neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL); however, these values (except EE and ADL) of ensiled leaves were significantly different among the clones. Mean CP, CA, and condensed tannin contents in dried leaves were 15.36, 9.21, and 1.75%, respectively; NDF, ADF, and ADL contents were 38.35, 35.49, and 12.08%, respectively. Mean total volatile fatty acids, *in vitro* organic matter digestibility (IVOMD), and metabolizable energy (ME) value in dried leaves were 95.26 mmol/L, 76.34%, and 10.77 MJ/kg, respectively, whereas, CO_2 and CH_4 production were 54.66 and 29.78 mmol/L, respectively. Buffering capacity and water-soluble carbohydrates varied among the pre-ensiled paulownia leaves, although their means were 395.66 mEq/kg DM and 86.63 g/kg DM, respectively. In ensiled leaves, the pH, lactic acid ratio, and acetic acid ratio were 4.98, 11.23, and 2.56%, respectively, and butyric acid was not detected in any of the silages. Mean values of IVOMD and ME in ensiled leaves were 72.30% and 9.93 MJ/kg, respectively. Dried paulownia leaves are a high-quality alternative forage and the ensiled form is of medium quality. Therefore, paulownia leaves could be used as an alternative roughage source for ruminants.

Keywords: alternative roughage, nutritive value, Paulownia tree leaf

***Corresponding author:**
huseyinipcak@gmail.com

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1. Introduction

As the increasing world population also increases the demand for food of animal origin, it also increases the need for alternative feed sources on agricultural lands as well as sufficient feed production; therefore, within the agricultural sector, the aim must be to improve the nutritional value of a product, create a sustainable and green agriculture, and reap more diverse products with a higher quality per unit area. For this, land management systems (i.e., agroforestry) (De Baets et al., 2007) is considered where agriculture, livestock, or agriculture + livestock are combined with planting various trees and where more than one product is obtained concurrently. Within these systems, the objective is to utilize the same land in multiple ways, obtain food and feed products simultaneously or consecutively, increase the yield from the land, improve the socioeconomic conditions, control illegal logging, and reduce erosion (Turna et al., 2014). These systems are applied as agrisilviculture (agriculture +

forestry), silvopastoral (forestry + livestock), agrosilvopastoral (agrisilviculture + silvopastoral), and the production of multipurpose trees (King, 1979; Jensen, 2016). Among these, the leaves of these multipurpose trees are used as fresh and green grass for ruminants during seasonal transitions and when roughage production is insufficient.

In Turkey, especially with silvopastoral systems, which are agroforestry systems with high-application potential, trees are planted for growing fodder leaves (Filiz and Tolunay, 2003). It has been reported that the leaves of 11 species of multipurpose trees (*Acacia* spp., *Albizzia procera*, *Calliandra calothyrsus*, *Dalbergia sissoo*, *Eucalyptus hybrida*, *Gmelina arborea*, *Leucaena leucocephala*, *Morus alba*, *Paulownia* spp., *Samanea saman*, and *Sesbania sesban*) can be used as a source of nutrition, especially for small ruminants, in terms of protein content (11-30%), organic matter digestion (49-51%), and metabolizable energy (ME; 6-7 MJ/kg) values (Dzowela et al., 1995; Datt et al., 2008; Woods, 2008; Vu et al., 2011).

The paulownia (*Paulownia* spp.) tree is an agroforestry tree used in silvorable or silvopastoral systems (Sonja, 2018). Its leaves are used as roughage in many countries, especially in China, South American countries, Japan, and Australia. The cultivation of this tree, which has the ability to grow very rapidly (1-2 m in the first year), for multicultural or biomass purposes, is ~2.4 mha worldwide (AFBI, 2008; Cheema et al., 2011). This tree is preferred because it grows between the rows of plants such as corn and cotton in the field without damaging those products and increases their yield. Indeed, in most countries, the flowers of the paulownia tree are used in beekeeping and cosmetics industries, while its large leaves (~0.5 m in the first year) are used for silage (Zhu et al., 1986; Mueller et al., 2001).

In Turkey, paulownia trees have been used in landscaping for several years; however, by cultivation, attempts have been made to produce multipurpose trees to obtain more products per unit of land within ten years (İncedemiroğlu and Çiçek, 2007); however, there have been no studies on the feed value of these leaves. Considering that 100 trees could be placed per hectare and one tree produces an average of 100 kg/year of leaves, it is possible to obtain 10 t/ha/year of fresh forage from leaves (Briggs, 2012). Hence, it can be suggested that they may have a high potential as an alternative roughage source.

This study aimed to determine the nutritional value and *in vitro* digestibility of paulownia tree leaves and demonstrate their potential as alternative source of roughage for ruminants.

2. Material and Methods

2.1. Feed materials

The paulownia leaves used in the study were taken from a special plantation area in İzmir-Menderes (38°15'14.4" N, 27°8'2.4" E) at the end of October 2018. The leaves were collected from one-year-old hybrid trees created by crossing different paulownia species—*Paulownia tomentosa, P. elongata, and P. fortunei*. These trees comprised C-125 (*P. elongata* × *P. elongata*), Caroline/CAR (*P. elongata* × *P. fortunei*), and TF-33 (*P. tomentosa* × *P. fortunei*) clones obtained from tissue cultures. Green leaves and their petiole were collected randomly from plants of the exact clone (10 trees per clone and approximately 1000 g of leaves of each tree) by hand. The samples were taken from the upper, middle, and lower branches of the tree [the average leave length (petiole not included) was 52.75 cm for C-125, 42.83 cm for TF-33, and 37.5 cm for CAR]. Samples collected from each tree were pooled and dried. Thus, the leaves of each tree were homogeneously used in chemical analysis. The chemical compositions of the paulownia leaves before ensiling are shown in Table 1.

2.2. Preparation of feed samples

The leaves brought to the laboratory were separated into use as dried and ensiled. Leaves (leaf + its petiole) were dried in an oven at 65 °C to obtain dried paulownia and then ground in a 1-mm mill. The leaves that were used for ensiled material were cut into 2- to 3-cm pieces using scissors and left to wither (~4 h). Withered leaves were placed in 50 × 70 cm airtight vacuum bags; the air inside the bags was vacuumed out, and the bags were wrapped tightly with duct tape. The ensiling period was 60 d.

Table 1 - Nutrient composition of fresh paulownia leaves (% DM)

Component	Clone			Mean
	C-125	CAR	TF-33	
DM	27.06	29.18	26.97	27.74
CA	8.55	9.00	10.50	9.35
HCl-insol.	1.06	1.02	1.02	1.03
CP	13.07	15.45	18.70	15.74
EE	4.38	4.41	3.12	3.97
CF	18.07	18.00	22.87	19.65
NfE	55.70	53.11	44.82	51.21
NDF	37.85	36.61	40.60	38.35
ADF	34.44	35.46	36.59	35.50
ADL	12.47	12.84	10.93	12.08
HEM	3.42	1.16	4.01	2.86
CEL	21.97	22.62	25.65	23.41
WSC (g/kg DM)	87.89	96.98	75.02	86.63
BC (mEq/kg DM)	391.72	383.83	411.43	395.66

C-125: *P. elongata* × *P. elongata*; CAR (Caroline): *P. elongata* × *P. fortunei*; TF-33: *P. tomentosa* × *P. Fortunei*.
DM - dry matter; CA - crude ash; HCl insol. - HCl insoluble CA; 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; HEM - hemicellulose; CEL - cellulose; WSC - water-soluble carbohydrates; BC - buffer capacity.

2.3. Chemical composition of paulownia leaves

The nutrient contents of the paulownia leaves [dry matter (DM), crude ash (CA), HCl insoluble ash (HCl-insol), crude protein (CP), ether extract (EE), crude fiber (CF), calcium, phosphorous, sugar (Luff Scroll), and starch) were determined using the Weende analysis method (Menke and Huss, 1975), and the CF contents using the fiber bag analysis technique. Organic matter (OM = DM − CA) and nitrogen-free extract (NfE = DM − [CA + CP + EE + CF]) contents of the leaves were calculated. The Ca and P contents of leaves were calculated according to the permanganometric and spectrophotometric (Ultraspec 2100 pro, USA) methods, respectively. The contents of the cell walls of the leaves [neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)] were determined according to the fiber bag system (Gerhardt, DE) developed as a result of the modification of the Van Soest analysis method (Goering and Van Soest, 1970). Sodium sulfide was used for NDF analysis. The hemicellulose (HEM) contents of leaves were calculated from the result of NDF − ADF, and the cellulose (CEL) content from that of ADF − ADL. The condensed tannin (CT) contents of leaves were determined according to the butanol–HCl method (Makkar et al., 1995). Anonymous (1986) was used to determine water-soluble carbohydrate content (WSC) in spectrophotometer by an antron-thiourea method, and the Playne and McDonald (1966) method was used for determining the buffer capacity (BC). The pH of ensiled leaves was measured using the Hanna HI2211 digital pH meter, and organic acids [lactic (LA), acetic (AA), and butyric (BA)] were created by distillation (DLG, 1987; Naumann und Bassler, 1993; Kılıç, 2010). In this method, the ensiled feed is extracted with water for one night and then filtered. The filtrate is mixed with solutions of 20% CaO and 20% $CuSO_4$. After 1 h, the liquor is filtered again, and then 48% H_2SO_4 is added to it for distillation. The first and subsequent distillation products (in the first 20 and the next 10 min) are recorded as D1 and D2. The D3 distillation is also obtained after adding chromic acid and pure water. The distillates are titrated in a 0.05 N NaOH solution and organic acids (lactic, acetic, and butyric) are calculated based on values obtained in titration.

2.4. *In vitro* rumen fermentation of paulownia leaves

The gas production technique was used to determine the *in vitro* values of leaf organic matter digestibility (OMD) and ME (Menke and Steingass, 1988). Using this method, the net gas production

(GP) of the feed was taken over 3, 6, 12, 24, 48, 72, and 96 h as a basis, and the *in vitro* organic matter digestibility (IVOMD) and ME values were calculated as follows:

$$IVOMD\ (\%) = 15.38 + 0.8453 \times GP + 0.0595 \times CP + 0.0675 \times CA$$

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1357 \times GP + 0.0057 \times CP + 0.0002859 \times EE^2,$$

in which GP = gas production after 24 h of fermentation (mL), CP = crude protein (g/kg DM), EE = ether extract (g/kg DM); and CA = crude ash (g/kg DM).

In the present study, the rumen content used was taken from healthy cows with unknown dietary at a slaughterhouse to replace cannulated animals in a trial evaluating feedstuffs (Lutakome et al., 2017). Accordingly, the fresh rumen content was immediately filled into thermos flasks (within 1 h of collection). The pH in the rumen fluid was measured using the Basic PB-20 digital pH meter (Sartorius, DE) and dipping directly into the rumen fluid. The Agilent Technologies 6890N and Stabilwax-DA gas chromatograph (30 m, 0.25 mm ID, 0.25 um df. max. temp: 260 °C. Cat. 11023) were used for analysis of the total volatile fatty acids (TVFA) in the rumen fluid [AA, BA, propionic acid (PA), valeric acid (VA), isovaleric (IVA), and isobutyric (IBA) acids] (Wiedmeier et al., 1987). Carbon dioxide (CO_2) and methane (CH_4) gases that were produced *in vitro* by fermentation were calculated using the following equations with the rumen fluid obtained after incubating for 24 h with VFA (Blümmel et al., 1997; Blümmel et al., 1999). The nitrogen content of ammonia (NH_3-N) in the rumen fluid was determined according to the recommendations of Blümmel et al. (1997).

$$CO_2\ (mmol\ L^{-1}) = (AA/2) + (PA/4) + (1.5 \times BA)$$

$$CH_4\ (mmol\ L^{-1}) = (AA + [2 \times BA]) - CO_2$$

2.5. Statistical analyses

Statistical analyses of the obtained data were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Data were checked for normality, using the Kolmogorov-Smirnov or Shapiro-Wilk tests, and for homogeneity of variances, using Levene's test. One-way analysis of variance (ANOVA) was applied to determine the difference between the mean values of groups. The post-hoc Duncan test was used to compare means when ANOVA was significant ($P<0.05$).

The mathematical model of the trial plan was:

$$Y_{ij} = \mu + \alpha_i + e_{ij},$$

in which μ = expected population mean, α_i = effect of clones in dried and ensiled paulownia leaves, and e_{ij} = experimental error.

3. Results

3.1. Nutritional compositions of dried and ensiled paulownia leaves

There were significant differences in the CA, CP, EE, CF, NfE, and sugar values of dried leaves of clones ($P<0.05$), with the highest values (except EE and NfE) found in TF-33 (Table 2). There were also differences in Ca and P contents ($P<0.05$), with the lowest values found in C-125. The highest Ca value was detected in TF-33, and that of P was in CAR. The CT values of clones also differed from 1.36 to 2.02%, with the lowest CT found in TF-33 and the highest in C-125. On the other hand, there was no difference in the cell wall components of the clones, which ranged from 37.85 to 40.60% of DM for NDF, 34.44 to 36.59% of DM for ADF, and 10.93 to 12.84% of DM for ADL ($P>0.05$).

The DM content in the ensiled leaves was between 23.76 and 27.81% and was lower than that in the pre-ensiled leaves (Table 3). Although the greatest DM loss was in the TF-33 clones, the highest CA (10.81% of DM) and highest CP (14.65% of DM) values were determined in these leaves ($P<0.05$). The EE values in leaves ranged from 3.25 to 3.52% of DM with no differences among them ($P>0.05$); however, the CF values ranged from 20.48 to 26.08% of DM in the clones, with the highest value found

Table 2 - Nutrient composition of dried paulownia leaves (% DM)

Component	Clone			Mean	SEM	P-value
	C-125	CAR	TF-33			
Crude nutrients						
DM	92.05b	92.24a	92.29a	92.18	0.037	0.019
CA	8.55b	9.00b	10.50a	9.21	0.342	<0.001
CP	13.07c	15.45b	18.71a	15.36	0.898	<0.001
EE	4.38a	4.41a	3.12b	3.97	0.270	<0.001
CF	18.07b	18.00b	22.87a	19.64	1.060	0.021
NfE	55.70a	53.11a	44.82b	51.21	2.111	0.006
Sugar	4.41c	5.47b	6.49a	5.46	0.380	<0.001
Cell-wall contents						
NDF	37.85	36.61	40.60	38.35	0.838	0.096
ADF	34.44	35.46	36.59	35.49	0.696	0.563
ADL	12.47	12.84	10.93	12.08	0.483	0.268
HEM	3.42	1.16	4.01	2.86	1.046	0.613
CEL	21.97	22.62	25.65	23.41	0.778	0.058
Minerals						
Ca	1.32c	1.88b	2.02a	1.74	0.107	<0.001
P	0.22c	0.87a	0.37b	0.49	0.125	<0.001
CT	2.02a	1.85b	1.36c	1.75	0.100	<0.001

DM - dry matter; CA - crude ash; 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; HEM - hemicellulose; CEL - cellulose; CT - condensed tannin; nd - not defined; SEM - standard error of the mean.
Values with different letters within the same row are significantly different at P<0.05.

Table 3 - Nutrient composition and fermentation characteristics of ensiled paulownia leaves (% DM)

Component	Clone			Mean	SEM	P-value
	C-125	CAR	TF-33			
Crude nutrients						
DM	27.81a	27.20a	23.76b	26.26	0.428	<0.001
CA	9.88b	10.02b	10.81a	10.21	0.108	<0.001
CP	10.78b	15.98a	14.65a	13.49	0.570	<0.001
EE	3.52	3.41	3.25	3.39	0.126	0.694
CF	26.08a	20.48b	22.66b	22.83	0.633	<0.001
NfE	42.66a	42.22a	35.66b	40.18	0.877	<0.001
Cell-wall contents						
NDF	41.64a	36.50b	40.18a	39.22	0.649	<0.001
ADF	39.17a	34.80b	35.87b	36.92	0.586	<0.001
ADL	13.89a	14.13a	11.33b	13.09	0.474	0.020
HEM	2.22b	1.71b	4.09a	2.56	0.357	0.019
CEL	25.41	20.94	22.89	23.51	0.751	0.050
CT	1.86a	1.65b	1.26c	1.59	0.088	<0.001
pH	5.02	4.84	5.07	4.98	0.050	0.128
LA	9.24b	11.45a	12.99a	11.23	0.517	0.004
AA	1.68b	1.89b	4.10a	2.56	0.292	<0.001
BA	0.00	0.02	0.00	0.01	0.007	0.452
WSC (g/kg DM)	53.85	56.94	50.69	53.83	2.008	0.414

DM - dry matter; CA - crude ash; 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; HEM - hemicellulose; CEL - cellulose; CT - condensed tannin, LA - lactic acid; AA - acetic acid; BA - butyric acid; WSC - water-soluble carbohydrates; SEM - standard error of the mean.
Values with different letters within the same row are significantly different at P<0.05.

in C-125 (P<0.05). Furthermore, although all ensiled leaves had lower CT values than dried samples, there was also a significant difference between the CT values of the leaves (1.26-1.86%), and the lowest value was found in TF-33 (P<0.05). Moreover, there were some differences in cell wall components of the clones (P<0.05). Accordingly, we found the lowest value of NDF in CAR (36.50% of DM) and of ADL in TF-33 (11.33% of DM) and the highest value of ADF in C-125 (39.17% of DM); however, there was no difference between the HEM and CEL values in the clones.

On the other hand, there was no difference in pH among the clones (P>0.05), which ranged between 4.84 and 5.07; however, the differences in the amount of organic acids (except BA) were significant (P<0.05), with the higher value of LA found in CAR (11.45% of DM) and TF-33 (12.99% of DM) when compared with that in C-125 (P<0.05). Besides, the highest AA value of the ensiled leaves was found in TF-33 (4.10% of DM) when compared with that in the others (P<0.05). Butyric acid was not determined in any sample (P>0.05); however, the WSC values in the ensiled leaves were similar (P>0.05) and ranged between 50.69 and 56.94 g/kg DM.

3.2. Rumen fermentation of dried and ensiled paulownia leaves

The effect of dried leaves of paulownia clones on rumen fermentation was significant (P<0.05) (Table 4). The highest TVFA and highest PA were found in TF-33 (95.18 and 20.99 mmol/L, respectively), although there was no difference in AA and BA among the clones (P>0.05). pH values ranged between 6.57 and 6.72, with the lowest value found in TF-33 (P<0.05). The highest NH_3-N values in the dried leaves were found in C-125 (35.29%) and TF-33 (36.20%) and the lowest in CAR (33.29%); however, we observed that these values were lower in the ensiled leaves and there was no difference among the clones in any of these parameters, with the exception of PA and VA (P>0.05).

The effect of clones on *in vitro* gas production, IVOMD, and ME values in the dried and ensiled paulownia leaves was significant (P<0.05) (Table 5). Accordingly, gas production in the dried and ensiled leaves increased with the incubation period and varied on average within the range of 17.12 to

Table 4 - Rumen fermentation from dried and ensiled paulownia leaves

		Clone			Mean	SEM	P-value
		C-125	CAR	TF-33			
Dried							
	TVFA	91.15b	90.43b	95.18a	95.26	0.910	0.040
	Acetic	54.18	53.26	54.88	54.11	0.497	0.466
	Propionic	18.78b	18.48b	20.99a	19.42	0.435	0.005
Volatile fatty acids (mmol/L)	Butyric	14.84	15.04	15.61	15.16	0.175	0.189
	Isobutyric	0.64b	0.69ab	0.78a	0.70	0.025	0.039
	Valeric	0.89	0.93	0.96	0.92	0.024	0.527
	Isovaleric	1.82b	2.04a	1.96ab	1.94	0.039	0.040
	pH	6.72a	6.64b	6.57b	6.65	0.023	0.006
	NH_3-N (%)	35.29a	33.29b	36.20a	34.93	0.485	0.010
Ensiled							
	TVFA	84.94	84.29	86.72	85.32	0.691	0.379
	Acetic	51.06	51.24	52.27	51.52	0.400	0.475
	Propionic	16.73ab	15.73b	17.45a	16.64	0.297	0.026
Volatile fatty acids (mmol/L)	Butyric	14.01	14.03	13.77	13.94	0.169	0.834
	Isobutyric	0.63	0.65	0.69	0.66	0.020	0.536
	Valeric	0.79b	0.89a	0.89a	0.86	0.019	0.031
	Isovaleric	1.72	1.74	1.65	1.70	0.034	0.586
	pH	6.79	6.77	6.68	6.75	0.023	0.084
	NH_3-N (%)	30.27	30.22	31.12	30.53	0.341	0.544

TVFA - total volatile fatty acids; SEM: standard error of the mean.
Values with different letters within the same row are significantly different at P<0.05.

70.01% and 15.30 to 65.20%, respectively, although the differences were significant during all times, except from 3 to 6 h for the dried leaves and 3 h for the ensiled leaves. The lowest and highest IVOMD values were found in C-125 for both the ensiled and dried leaves (69.47 and 72.53%, respectively) and TF-33 for ensiled and dried leaves (74.35 and 81.02%, respectively). Similarly, the highest ME value was found in TF-33 for dried (11.05 MJ/kg DM) and ensiled (10.24 MJ/kg DM) leaves; the lowest value was determined in C-125 for both dried (10.47 MJ/kg DM) and ensiled leaves (9.70 MJ/kg DM).

The effect of clones on *in vitro* CO_2 and CH_4 production in dried and ensiled paulownia leaves was not significant (P>0.05) (Table 6). Accordingly, CO_2 production was between 53.81 and 56.10 mmol/L in dried leaves and 50.60 and 51.16 mmol/L in ensiled leaves. In addition, CH_4 was detected in both the dried and ensiled leaves at between 29.53 and 30.00 and 28.35 and 28.70 mmol/L, respectively.

Table 5 - *In vitro* gas production (mL/200 mg DM), IVOMD (%), and ME values (MJ/kg DM) of dried and ensiled paulownia leaves

		Clone			Mean	SEM	P-value
		C-125	CAR	TF-33			
Dried							
	3 h	17.93	16.83	16.60	17.12	0.287	0.116
	6 h	25.33b	27.57a	29.07a	27.29	0.639	0.013
	12 h	36.93c	41.83b	43.80a	40.86	1.039	<0.001
Gas production (mL/200 mg DM)	24 h	50.20b	51.50b	54.30a	52.00	0.637	<0.001
	48 h	59.87b	60.87b	64.10a	61.61	0.664	<0.001
	72 h	67.77ab	66.50b	69.40a	67.89	0.485	0.016
	96 h	68.93b	68.33b	72.77a	70.01	0.734	0.001
	IVOMD	72.53c	75.47b	81.02a	76.34	1.256	<0.001
	ME	10.47c	10.78b	11.05a	10.77	0.088	0.001
Ensiled							
	3 h	15.17	15.77	14.97	15.30	0.241	0.424
	6 h	22.00b	26.33a	24.60a	24.31	0.679	0.003
	12 h	34.10b	38.33a	36.67a	36.37	0.671	0.004
Gas production (mL/200 mg DM)	24 h	47.40b	46.33b	50.03a	47.92	0.664	0.031
	48 h	58.67a	55.17c	57.10b	56.98	0.537	0.001
	72 h	64.33a	61.50b	63.23ab	63.02	0.497	0.031
	96h	66.80a	63.33b	65.47a	65.20	0.545	0.003
	IVOMD	69.47c	72.09b	74.35a	72.30	0.906	0.002
	ME	9.70b	9.85b	10.24a	9.93	0.095	0.022

IVOMD - *in vitro* organic matter digestibility; ME - metabolizable energy; SEM - standard error of the mean.
Values with different letters within the same row are significantly different at P<0.05.

Table 6 - Carbon dioxide and CH_4 gas production (mmol/L) from dried and ensiled paulownia leaves

	Clone			Mean	SEM	P-value
	C-125	CAR	TF-33			
Dried						
CO_2	54.05	53.81	56.10	54.66	0.469	0.064
CH_4	29.82	29.53	30.00	29.78	0.212	0.722
Ensiled						
CO_2	50.72	50.60	51.16	50.83	0.397	0.871
CH_4	28.35	28.70	28.66	28.57	0.209	0.803

SEM - standard error of the mean; P<0.05.

4. Discussion

4.1. Nutritional compositions of dried and ensiled paulownia leaves

In the study, the chemical composition in terms of CA, CP, CF, and Ca in dried paulownia leaves differed among clones, and TF-33 had the highest values. Compared with previous studies about *Paulownia* spp. leaves, the CP content of dried leaves in our study was higher than that in Varlyakov et al. (2013) and Gutiérrez et al. (2015), but lower than that in Mueller et al. (2001). The differences in nutrients can be attributed to species, age, and climatic conditions. The chemical composition of foliage belonging to this clone is similar to that found in some of the studies made using acacia leaf (Abdulrazak et al., 2000a,b; Mokoboki et al., 2005) and are compatible with the results of several studies on different tree leaves, such as oak, mulberry, eucalyptus, and exotic wood species in Turkey (Ataşoğlu et al., 2010a,b; Canbolat, 2012; Güven, 2012; Akçil and Denek, 2013; Kurt and Öztürk, 2018). On the other hand, the Ca and P contents in leaves (excluding the CAR clone) exceeded the desired ratio of 2:1, but this problem could be easily corrected using cautious mineral supplementation. However, the CT in the dried leaves differed among clones, and C-125 had the highest value, whereas TF-33 had the lowest. This value in some tree leaves has been reported to vary between 0.1 and 16% (Abdulrazak et al., 2000a,b; Mokoboki et al., 2005; Ataşoğlu et al., 2010a,b; Elahi, 2010; Canbolat, 2012; Akçil and Denek, 2013; Kurt and Öztürk, 2018). Although a low condensed tannin level of 2-3% in ruminant nutrition has a beneficial effect because it decreases protein degradation in the rumen (Barry, 1987; Canbolat, 2012), a high level (>5%) negatively affects protein digestion and microbial and enzymatic activities (Kumar and Singh, 1984); therefore, it can be reported that the CT values of all clone leaves are suitable for rumen fermentation.

In ensiled leaves, the clone was important in DM, CA, CP, CF, and NfE. Clones C-125 and CAR had the highest value for DM, whereas CAR and TF-33 had the highest value for CP. The CP value of leaves belonging to these clones was higher than that in corn silage and lower than that in meadow grass silage (DLG, 1991; NRC, 2001). In our study, it was observed that ensiling suppressed the CT value of leaves belonging to all clones. The lowest and highest CT contents were respectively found in TF-33 and C-125, again. According to data, it can be stated that paulownia leaves are sufficient in crude nutrients, especially for small ruminants, and could be an alternative roughage source.

Furthermore, there was no difference among clones for NDF, ADF, and ADL in dried form. The difference was significant in the ensiled form, and CAR had the lowest value for NDF, but ADF was the lowest in CAR and TF-33. The NDF and ADF contents obtained for dried paulownia leaves were similar to the results of Mueller et al. (2001). Neutral detergent fiber was lower, and ADF was higher than the values of *P. elongata* leaves found in Gutiérrez et al. (2015). However, dried leaves of all clones were lower in those ratios than that found in the leaves of acacia and oak species (Mokoboki et al., 2005; Elahi, 2010) and similar to that found in Chitra and Balasubramania (2016). Our results are also consistent with those of many studies on the leaves of different tree species (e.g., oak, mulberry, eucalyptus, exotic tree species) in Turkey (Ataşoğlu et al., 2010a,b; Canbolat, 2012; Kurt and Öztürk, 2018). This can be attributed to species, age, and climatic conditions. In the present study, the HEM ratio in leaves belonging to both forms, in particular, was quite low because the NDF and ADF ratios in the leaves were very similar. This was attributed to the fact that the paulownia tree used was a hybrid plant that grew very fast in the early years. Indeed, Jung and Allen (1995) reported that the biotechnological processes applied to plants can cause differences in their cell wall biosynthesis and that these analyses are insufficient in fast-growing hybrids, such as paulownia tree. Otherwise, in the present study, the average cell wall component was lower in dried leaves than in silages, because the leaves deteriorated into botanical fractions in the silage compared with that before being ensiled. Indeed, the CP content was lower in the ensiled than in the dried leaves, which was the result of leaf damage during fermentation such that, when the silage was opened, the leaf:stem ratio shifted toward the stem. In addition, cell wall results showed that the paulownia leaf silage had low NDF but high ADF contents compared with corn silage, but low NDF and ADF contents compared with meadow grass

silage (NRC, 2001; McDonald et al., 2010); therefore, we believe that paulownia leaves are sufficient in terms of their cell wall contents and that drying or ensiling does not affect these parameters.

There was a difference among clones for LA and AA, whereas pH, BA, and WSC were similar. According to the data on silage fermentation, the pH value was slightly higher than desired (3.5-4.5) in good-quality silages; however, these high pH values were not significant. Lactic acid is responsible for a low pH and the ratio of this acid should constitute 65-70% of the total silo acids for a successful fermentation (Kung Jr., 2010; Kılıç, 2010). The LA ratios of total acids in ensiling of C-125, CAR, and TF-33 leaves were 85, 86, and 76%, respectively. However, the difference among clones for AA was quite high, and the highest value was in TF-33. A high AA ratio in the silages has been associated with the converting carbohydrates or lactate in the environment to PA or AA or the development of heterofermentative lactic acid bacteria that produces AA (Ray and Daeschel, 1992; Filya and Sucu, 2005; Kung Jr., 2014). On the other hand, it was reported that there was a close relationship between AA and aerobic stability in some studies, because AA could play a critical role in inhibiting harmful microorganisms in the silage (Danner et al., 2003; Kung Jr., 2018). Therefore, the greatest aerobic stability between ensiled paulownia clones can be expected in TF-33.

Water-soluble carbohydrate rate was decreased in all ensiled leaves in proportion to fresh material. Nevertheless, the highest decrease was in CAR, C-125, and TF-33, and the WSC contents of all clones were found similar in ensiled form even if it was not the same initially. Baghdadi et al. (2016) reported that WSC helped to drop pH rapidly in silage in their study. Low buffer capacity also plays a critical role in the drop of pH (McDonald et al., 2010), explaining why C-125 had a lower pH than others, even if there is no difference between groups.

4.2. Rumen fermentation of dried and ensiled paulownia leaves

In our study, the rate of total VFA was the highest in TF-33 in dried form, whereas the values were similar in ensiled form. Total volatile fatty acids are the main products after microbial fermentation in the rumen (Castillo-González et al., 2014) and are evaluated in energy metabolism in ruminants (Wang et al., 2020). In the present research, TF-33 in dried form had the highest sugar content, and this value increased the TVFA rate by inducing fermentation in the rumen. However, there was no difference for TVFA in ensiled form, because sugar was used during silage fermentation. On the other hand, in dried and ensiled leaves, TF-33 had the highest value for propionic acid, while there was no significant difference among the clones for acetic and butyric acids. The reason why TF-33 had the highest propionic acid in silage form may be because it has the highest LA value as a fermentation characteristic, because propionic acid, one of the major TVFA, is also one of the major volatile acid produced by carbohydrates or LA fermentation (Wang et al., 2020). Therefore, it can be thought that the LA ratio of the silage changes the propionic acid value in the rumen. On the other hand, the type of clone used altered the value of rumen pH and rate of NH_3-N in dried leaves, whereas it was not important in ensiled form. When the present research was compared with previous studies on the effects of various tree leaves (*Azadirachta indica*, *Newbouldia laevis*, *Populus* L., *Quercus* L, *Robinia pseudocacia* L., *Fagos adsidue*, *Spondias mombin*) on the rumen characteristics, it was observed that the presence of tannin in the leaves changed the microflora and fermentation. Additionally, the rate of CP was also important for optimum fermentation due to the use of rumen microorganism N as a source of protein (Adelusi et al., 2016; Özdemir and Kaya, 2020). Furthermore, the difference was significant for IVOMD values of dried and ensiled leaves. Gutiérrez et al. (2015) reported lower IVOMD of *P. elongata* than that found in the present study for the dried leaves. The difference in these values between the two studies might have been a result of species. Moreover, some studies have reported that IVOMD values in different tree leaves were lower than the leaves of tree clones used in the present study (Kamalak et al., 2005; Ataşoğlu et al., 2010a,b; Canbolat, 2012; Güven, 2012). As shown in some studies, the digestibility values of leaves change according to chemical composition and secondary metabolite content of tree except for type. Abdulrazak et al. (2000a,b) reported that phenolic compounds have a negative effect on IVOMD. Indeed, in the present study, we observed that IVOMD increased with decreasing CT in both the dried and ensiled leaves. The difference was significant for ME values in dried and ensiled

paulownia leaves. According to data, the greatest rate of these parameters was detected in TF-33. It was also higher than the values that Gutiérrez et al. (2015) reported in their studies. However, the ME value in dried leaves was similar to that in other tree leaves, such as oak, mulberry, and eucalyptus (Ataşoğlu et al., 2010a,b; Canbolat, 2012; Güven, 2012; Akçil and Denek, 2013). When the ME value in dried leaves was compared with dry roughages, it was detected that it was higher than that in alfalfa hay, immature grass-legume hay, and grass hay. The ME value in ensiled leaves was also higher than in the ensiled triticale, grass, and corn (NRC, 2001).

In contrast, the clones show no effect on CO_2 and CH_4 gas production in both dried and ensiled form. Sallam et al. (2010) stated that plants rich in condensed tannins alter methanogenesis by reducing methanogen activity, indirectly affecting fiber digestion in rumen. In addition, another study reported that CH_4 production decreases with silage nutrition compared with nutrition in dry roughages, which was attributed to the formation of less AA during ruminal fermentation of silage feed (Morgavi et al., 2011; Meral and Biricik, 2013). This report supports the low AA ratio in the rumen of ensiled leaves compared with that in dried paulownia leaves. On the other hand, Hindrichsen et al. (2004) reported that feeds high in lignin suppress CH_4 production, and less AA is formed from the lack of lignin digestion in the rumen. It has also been reported that ADL is the carbohydrate form that suppresses CH_4 production the most, and as the amount of ADL in the ratio increases, the IVOMD value and CH_4 production decrease. This report was consistent with the high ADL content, low IVOMD, and low CH_4 values in ensiled leaves compared with those in dried paulownia leaves.

5. Conclusions

Ensiled and dried forms of paulownia (especially TF-33) leaves could be used as an alternative roughage source in ruminant feeding in nutrient composition, *in vitro* organic matter digestibility, metabolizable energy value, and rumen fermentation characteristics.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Data curation: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Formal analysis: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Funding acquisition: H. Özelçam, H.H. İpçak and S. Özüretmen. Investigation: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Methodology: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Project administration: H. Özelçam, H.H. İpçak and S. Özüretmen. Resources: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Software: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Supervision: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Validation: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Visualization: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Writing-original draft: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat. Writing-review & editing: H. Özelçam, H.H. İpçak, S. Özüretmen and Ö. Canbolat.

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