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Seasonal Variation in Chemical Composition, Ruminal Fermentation, and Biological Characteristics of *Paulownia shan tong*: In Vitro Potential Use by Sheep and Goats

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Abstract: This study was conducted to determine the seasonal variation (two seasonal harvests during winter and spring) in some chemical and biochemical characteristics of *Paulownia shan tong* leaves. To achieve this, we analyzed the chemical composition in terms of dry matter (DM), organic matter (OM), crude protein (CP), cell-wall content (neutral detergent fiber (NDF)), acid detergent fiber (ADF), acid detergent lignin (ADL), and lipids. The phenolic compounds (PC) and antioxidant activity (AA) of the leaves were also determined. In vitro gas production (GP) and nutrient degradability were evaluated using two ruminal-content donors (sheep and goats). Higher ($p < 0.05$) antioxidant activities were observed in the winter harvest of *P. shan tong*, while higher concentrations of OM (90.5%), lipids (3.02%), NDF (69.7%), ADF (54.8%), ADL (35.4%), total flavonoids (0.45 mg catechin equivalents/mg DM), and total phenolics (2.52 mg gallic acid equivalents/mg DM) were observed in the spring harvest. Higher asymptotic GP and rate of GP ($p < 0.05$) were observed when the leaves were incubated with rumen liquor of goats compared to sheep; however, higher ($p < 0.05$) lag time of GP and half-time of GP were observed with rumen liquor from sheep. Moreover, higher asymptotic GP, rate of GP, and half-time of GP were observed with the winter harvest of *P. shan tong*. Higher ($p < 0.001$) metabolizable energy, OM degradability, and microbial protein supplies were obtained when the leaves were incubated with rumen fluids from goats and from the winter harvest of *P. shan tong* compared to the spring harvest. In conclusion, based on the chemical composition and the kinetics of in vitro gas production, *P. shan tong* leaves seem to be characterized by a good nutritive value and could be considered as an alternative feed resource for ruminants.

Keywords: *Paulownia shan tong*; nutritional values; antioxidant activity; digestibility

1. Introduction

In Tunisia, especially in arid and semi-arid areas, livestock production plays significant socio-economic and ecological roles [1]. In these areas, the major constraint on small ruminant livestock is the scarcity and fluctuations in the quantity and quality of fodder resources

throughout the year [2]. Thus, an increase in animal density has been observed, which has led to the massive degradation of rangelands. The availability and quality of feed are the main factors conditioning animal production and limiting animal performance. From a nutritional point of view, trees and shrubs have proven to be very effective in covering ruminant needs at different times of the year, particularly for leguminous species [3–5]. However, to our knowledge, there is a scarcity of studies on the nutritional value of cultivated trees of exotic origin, such as *Paulownia*, in Tunisia. The bibliographic research shows the nutritional usefulness of this species in the diet of ruminant animals [6,7]. *Paulownia shan tong* is currently the most appropriate type of *Paulownia* for investment in invaluable timber plantations as well as in biomass plantations.

It is a fast-growing deciduous tree grown in many countries for wood production. The leaves of the *P. shan tong* tree contain considerable concentrations of protein (17.5%), calcium (2.1%), zinc (0.9%), phosphorus (0.6%), iron (0.6%), and cellulose (15.1%) [8]. Moreover, the leaves contain high concentrations of bioactive compounds, including phenolic acids and flavonoids [9]. These secondary metabolites, mainly flavonoids and tannins, are generally characterized by their multiple biological activities [10]. The concentration of the plant's secondary metabolites depends on the growth stage and environmental conditions. Plant secondary metabolites in *Paulownia* play an important role in ruminal fermentation and nutrient digestibility when fed to animals [8,11]. Flavonoids and tannins, at appropriate levels, positively improve ruminal fermentation and reduce methane production [12,13]. Tannins can also affect cell wall digestibility to a variable extent because the interactions between tannins and either digestive enzymes or feed components (protein, cell wall carbohydrates) vary with plant species, animal species, tannin levels, and possibly tannin structure [14]. Recently, Huang et al. [15] evaluated the nutritive value of *Paulownia* leaves as feed for ruminants and concluded that it had a good nutritive value and could be considered as a ruminant feed resource [8]. Huang et al. [9] and Puchalska et al. [11] stated that the inclusion of either fresh or ensiled *Paulownia* leaves decreased methane production and the number of methanogens and improved the measured ruminal fermentation parameters. Recently, Huang et al. [15] stated that ensiled *Paulownia* leaves at 60 g/kg DM reduced methanogenesis and beneficially modulated ruminal fermentation and biohydrogenation processes without a negative impact on the milk production performance of lactating dairy cows.

Using different sources of rumen fluid to inoculate the in vitro incubation cultures is a useful tool to examine possible differences in the ruminal microbial population and the digestive capacity of ruminant species [16]. Different ruminant species have different comparative abilities to digest feeds; therefore, examining the fermentative capacity of the same feed with microbial populations from sheep and goats is important. Furthermore, the harvesting season dramatically affects the nutritive value of plants [17]. Horst et al. [17] observed that the digestibility and the acetate-to-propionate ratio of eucalyptus foliage were higher during the winter than during autumn and spring without affecting methane emissions. Thus, the hypothesis of the present study is that different relationships exist between the nutritional value of *Paulownia* feed materials harvested during two different seasons as well as their content of secondary metabolites, which may impact their in vitro gas production (GP) and digestibility. The two sampling seasons (winter and spring) were selected first because they corresponded to seasons with significant variation in environmental characteristics and they are frequently used by animals looking for alternative feed resources. Second, the sampling seasons correspond to the pruning season (winter), and the beginning of a new vegetative cycle (spring). This difference in the vegetative cycle might affect the nutritional value of plant species [14]. The present trial was designed as a preliminary screening of the nutritive value of *P. shan tong* harvested during two different seasons (winter and spring) and the potential of its ruminal fermentation when incubated in vitro with rumen fluid collected from sheep or goats.

2. Materials and Methods

2.1. Plant Material

Leaves of *Paulownia shan tong* (*P. shan tong*) were collected in winter (January) and spring (March) from a private rangeland situated in the region of El Fahs. The sampling region, a semi-arid area with average annual precipitations of 440 mm, is located in the north of Tunisia at 195 m above sea level and at 36°22′27″ latitude and 9°54′23″ longitude. At the moment of sampling, trees were two years of age with 6 m in length and 10 cm in diameter. Trees were planted on a 3 ha area, 1.5 × 1.5 m apart and irrigated every 15 days. The cultivated area was subdivided into three main plots. The farmer's main objective was to simultaneously produce wood and forage for small ruminant species raised in the region. In order to increase biomass production and obtain expanded and large leaves, a very intensive tree pruning is conducted in winter during dormancy and leaf falling. The main trunk is kept at a very short height. The vegetative tree regrowth is regenerated three months later and is accelerated during the spring season (March–May) with the appearance of new branches, large leaves, and twigs. The first sampling corresponded to the pruning season (winter) and leaves were collected from pruned branches. The second sampling corresponded to the beginning of a new vegetative cycle (March). The sampling was conducted at 3 different tree levels at each harvest time (winter or spring) in three plots. A total of 12 trees was sampled from the three plots (4 trees/plot). Leaves from the same plot irrespective of the sampling level (high, medium, and low) were mixed and constituted one sample. At each sampling, three samples were obtained (the mixture of three levels and four trees at each plot). Afterward, samples were air-dried at room temperature (40 ± 2 °C) for one week, ground in a Retsch blender mill (Normandie-Labo, Normandy, France), and sieved through a 0.5 mm mesh screen to obtain a uniform particle size. The ground substrates were stored in a dark room at room temperature until further use.

2.2. Chemical Analysis

Dry matter (DM), ash, and crude protein (CP) contents were determined according to AOAC [18]. A total lipid extraction was performed using the Soxhlet extraction method [18]. In short, dried and finely ground plant materials were mixed with boiling water to inactivate tissue phospholipases. The final extract was evaporated under reduced pressure in a Heidolph rotary evaporator (Schwabach, Germany), and the total lipids were determined. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were measured following the procedure proposed by Van Soest et al. [19] using a fiber analyzer (ANKOM 2000, ANKOM Technology, Macedon, NY, USA). The estimated parameters were determined in terms of % DM, as follow:

$$\text{Hemicellulose (HC, \%)} = \%\text{NDF} - \%\text{ADF} \tag{1}$$

$$\text{Crude cellulose (CB, \%)} = \%\text{ADF} - \%\text{ADL} \tag{2}$$

$$\text{Cell contents (CC, \%)} = 100\% - \%\text{NDF} \tag{3}$$

$$\text{Glucids (\%)} = \%\text{MO} - \%\text{ Lipids} - \%\text{CP} - \%\text{ADL} \tag{4}$$

2.3. Phenolic Compounds

2.3.1. Extract Preparation

Dried and ground samples of both studied species (1 g) were suspended in 20 mL of distilled water and robustly vortexed for 10 min with a rotator. The mixture was then kept for 24 h at room temperature (40 ± 2 °C) and then submitted for ultrasonic extraction for 20 min. Filtration was then carried out with a solid-phase extraction system and filtered through Whatman #1 filter paper (Bärenstein, Germany). The aqueous extracts were subsequently evaporated and dried under reduced pressure. The dried extracts were weighed to determine the extraction yield. The dried extracts were then redissolved with 3 mL of distilled water, collected, and conserved at 4 °C for further analysis.

2.3.2. Total Phenolic Contents

The total phenolic content (TP) of the ethanolic extracts was determined using the Folin–Ciocalteu method [20]. Approximately 0.5 mL of the aqueous extract was mixed with 0.5 mL freshly diluted Folin–Ciocalteu (10%) reagent and 1 mL of 7.5% sodium carbonate solution. After incubation for 1 h in the dark at room temperature (40 ± 2 °C), the absorbance of the extract was measured at 760 nm using a spectrophotometer (Jenway spectrophotometer monofaisceau UV/visible model 7315). The calibration curve for TP was established using gallic acid as a standard solution and the results were expressed as milligrams of gallic acid equivalents per g dry weight of the extract (mg gallic acid equivalents (GAE)/g DM).

2.3.3. Total Flavonoid Contents

The total flavonoid content (TF) of the extracts was determined according to the method proposed by Chang et al. [21]. One mL of the aqueous extracts was mixed with 1 mL of $AlCl_3$ 2% methanolic solution and vigorously shaken. After incubation for 15 min, the absorbance was measured at 415 nm. The calibration curve for total flavonoids was established using quercetin as a reference standard solution. The total flavonoid contents were expressed as milligrams of quercetin equivalents per g dry weight of the extract (mg QE/g DM).

2.3.4. Total Condensed Tannins

The vanillin–HC1 method of Broadhurst and Jones [22] was used. Briefly, 0.5 mL of the diluted extracts were placed into test glass tubes and added to 3 mL of vanillin reagent (4% *w*/*v*) and 1.5 mL concentrated HCl. The mixture was vigorously shaken. After incubation at room temperature (40 ± 2 °C) for 15 min, the absorbance was measured at 500 nm. The calibration curve for total condensed tannins was established using catechine as a reference standard. The total condensed tannin contents (TCT) were expressed as milligrams of catechin equivalents per g dry weight of the extract (mg EC/g DM).

2.4. Antioxidant Activity

2.4.1. DPPH-Scavenging Activity

The free-radical-scavenging capacity of aqueous extracts of leaves of *P. shan tong* was analyzed using a synthetic free-radical compound [1,1-diphenyl-2-picrylhydrazyl (DPPH)] according to the method described by Brand-Williams et al. [23] with some modifications. Briefly, 1 mL of aqueous extracts was added to 1 mL of DPPH ethanolic solution 0.06 mM (2.4 mg/100 mL). The mixture was vigorously shaken and saved at room temperature (40 ± 2 °C) for 30 min. The absorbance was then measured at 517 nm using a UV-visible spectrophotometer against the control (1 mL of distilled water and 1 mL of DPPH solution). The DPPH-radical-scavenging activities were calculated as follows:

$$\mathrm{PI}\ (\%) = \left[\frac{(\boldsymbol{Abs\ control} - \boldsymbol{Abs\ test})}{\boldsymbol{Abs\ control}}\right] \times \mathbf{100} \quad (5)$$

The antioxidant activity of the sample was expressed as the inhibitory concentration (IC_{50}) of the sample needed to inhibit 50% of the DPPH radicals.

2.4.2. ABTS-Scavenging Activity

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was based on the procedure described by Re et al. [24] The radical cation ABTS was obtained by mixing an equal volume (0.5 mL) of a solution of potassium persulfate $K_2S_2O_8$ of 3 mM (13.2 mg/mL) with a stock solution of ABTS at 8 mM (8 mg/mL). The mixture was reacted in the dark at 4 °C during 16 h. Then it was diluted with phosphate buffer (0.2 M, pH 7.4) containing 150 mM NaCl to obtain an absorbance of 0.7 at 734 nm. The diluted ABTS solution (2850 µL) was mixed with 150 µL of sample extract or trolox standard. The mixture was left to stand

at room temperature (40 ± 2 °C) in the dark for 15 min, and then the absorbance was measured at 734 nm. The results were expressed as percent inhibition (PI %), estimated as follow:

$$\text{PI (\%)} = \left[\frac{(\boldsymbol{Abs\ control} - \boldsymbol{Abs\ test})}{\boldsymbol{Abs\ control}}\right] \times 100 \quad (6)$$

2.5. Kinetics of In Vitro Gas Production

The rumen fluid used for the in vitro incubations was withdrawn from four adult local sheep (Queue Fine de l'Ouest) and four adult local goats (Arbi goats) from northwestern Tunisia. The rumen fluid was collected separately in the local slaughterhouse. Slaughtered goats were raised under an extensive production system, grazing in mountainous rangelands dominated by tanninferous species. However, slaughtered sheep were raised in a feedlot system. About 15 min post-slaughter, the rumen wall of each animal was cut open with a kitchen knife to collect the contents into pre-warmed thermal flasks and immediately transported to the laboratory, where it was continuously flushed with CO_2. The ruminal digesta collected was then strained through four layers of cheesecloth to remove large feed particles and the particulate materials were squeezed to obtain microbes attached to feed particles and kept at 39 °C under a CO_2 atmosphere. The rumen fluid collected separately from sheep and goats was mixed to obtain a single inoculum for each animal species. A culture medium containing macro and micro mineral solutions, resazurin, and bicarbonate buffer solution was prepared [25]. The medium was kept at 39 °C and saturated with CO_2. The rumen fluid was then diluted in the culture medium at a proportion of 1:2 (*v*:*v*). For each incubation run and source of rumen liquid (sheep/goat), the initial pH of the medium with inoculum was 6.8 to 6.9 ± 0.1 to keep the optimal activity of ruminal microbes [26]. Plant material samples (300 mg) collected in winter and spring were incubated separately using the two inoculate sources. They were weighed in a glass syringe (capacity 100 mL), added to 30 mL of the diluted rumen fluid, and incubated in a water bath (39 °C). All treatments were incubated with two replicates per run. A total of three runs was performed over three consecutive weeks for each rumen liquid source (sheep or goat). During each week, an incubation run with sheep rumen liquid and another with goat rumen liquid were carried out simultaneously. The volume of gas produced in the syringes was measured every 2 h (from 0 to 48 h). The data were fitted to the model proposed by France et al. [27]:

$$\mathrm{Y} = \boldsymbol{A}\left(\boldsymbol{1} - \boldsymbol{e}^{-\boldsymbol{c}[\boldsymbol{t} - \boldsymbol{L}]}\right), \quad (7)$$

where Y (mL/g) denotes the volume of cumulative gas produced at time t, A (mL/g) is the asymptotic GP, c (/h) is the fractional fermentation rate, and L (h) is the lag time. For each rumen liquid source (sheep or goats) and each substrate (three samples of *P. shan tong* from each season), two syringes were incubated in a single run. Two syringes containing inoculum (two per inoculum) and buffer but no feed (blanks) were included to establish the baseline fermentation GP. The values of the two syringes for each inoculum and substrate were considered analytical repetitions and averaged for subsequent statistical analysis. The slaughtered animals (sheep/goat) used as donor animals at each incubation run were issued from the same flock. This means that the donor animals used in the different runs were maintained in the same conditions and fed the same diet.

2.6. Calculations

The digestibility of organic matter (dOM) was estimated according to the formula proposed by Menke and Steingass [28]:

$$\text{dOM (\%)} = 14.88 + 0.889 \times \text{GP} + 0.45 \times \text{CP} + 0.0651 \times \text{MM} \quad (8)$$

Metabolizable energy (ME) was calculated according to Menke et al. [29]:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \times \text{G}_{24} + 0.057 \times \text{CP} \quad (9)$$

G_{24} is the volume of gas produced (mL/200 mg DM) at 24 h incubation, CP is the crude protein (g/100 g DM), and MM is the mineral matter (g/100 g DM).

The protein (in terms of PDI or protein digested in the small intestine) and energy (in terms of forage units for lactation (UFL) or for meat production (UFV)) value of *P. shan tong* were assessed according to the INRA feed evaluation system [30] and estimated from the feed characteristics (chemical composition and in vitro digestibility parameters) determined in our study using the INRAtion software.

2.7. Statistical Analyses

All data on chemical composition were subjected to analysis of variance using a one-way model with the fixed effect of season (winter vs. spring). The data on gas-production kinetics, digestibility, and feeding (energy and protein) value were subjected to analysis of variance using a 2 × 2 factorial model with the fixed effects of season (winter vs. spring), animal species (goat vs. sheep), and their interaction. For all the analyses, the experimental unit was the independent sample collected in each season, thus resulting in three replicates per season. The PROC GLM procedure of SAS (v. 9.2; SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses. Significance was declared at $p < 0.05$. All chemical composition and in vitro fermentation data were used to perform hierarchical cluster analyses using the unweighted pair group method with arithmetic mean (UPGMA) method and both the Euclidean distance and the Bray–Curtis as similarity indices. One analysis was applied to examine how the available data could discriminate both harvest seasons. In a subsequent cluster, the data matrix was used to analyze the differences between harvest seasons and ruminant species (sheep vs. goat) in the ruminal fermentation of *Paulownia* foliage.

3. Results

3.1. Chemical Composition and Secondary Metabolites

The concentrations of OM ($p = 0.006$), lipids ($p < 0.001$), NDF ($p < 0.001$), ADF ($p = 0.003$), and ADL ($p = 0.014$) differed between harvest seasons (Table 1). The winter harvest showed lower concentrations compared to the spring harvest of OM (89.8 vs. 90.5%), lipids (2.74 vs. 3.02%), NDF (55.6 vs. 69.7), ADF (37.9 and 54.8%), ADL (26.3 vs. 35.4%), and CB (19.6 vs. 11.58%). However, no differences between the two harvests were observed for the concentrations of CP and hemicellulose. Cell contents and glucids were higher in leaves harvested in winter as compared with those sampled in spring.

Table 1. Seasonal variation in chemical composition (% DM) of *Paulownia shan tong* harvested in winter and spring.

Items	Winter	Spring	SEM	*p* Value
OM	89.8 ^{b}	90.5 ^{a}	0.05	0.006
CP	19.4 ^{b}	19.8 ^{a}	0.23	0.335
Lipids	2.74 ^{b}	3.02 ^{a}	0.00	<0.001
NDF	55.6 ^{b}	69.7 ^{a}	0.55	<0.001
ADF	37.9 ^{b}	54.8 ^{a}	1.86	0.003
ADL	26.3 ^{b}	35.4 ^{a}	1.53	0.014
HC [1]	17.8	14.9	1.70	0.298
CB [2]	11.58 ^{b}	19.46 ^{a}	1.76	0.034
CC [3]	44.37 ^{a}	30.27 ^{b}	0.546	<0.001
Glucids [4]	41.37 ^{a}	32.35 ^{b}	1.604	0.017

Means in the same row with different superscripts differ, $p < 0.05$. SEM = Standard error of the mean. OM, Organic matter; CP, Crude protein; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; ADL, Acid detergent lignin; [1] HC, Hemicellulose (%DM) = %NDF − %ADF; [2] CB, Crude cellulose = %ADF − %ADL; [3] CC, Cell contents = 100% − %NDF; [4] Glucids, %MO − % Lipids − %CP − %ADL.

Although the harvesting season had no effect on the concentration of CT (Table 2), *P. shan tong* during winter showed a higher IC50_DPPH (0.32 vs. 0.22 mg/mL) ($p = 0.009$) and

IC50_ABTS+ (0.41 vs. 0.31 mg/mL) (p = 0.002) compared to the spring harvest, which had higher concentrations of TF (0.36 vs. 0.45 mg catechin equivalents (CE)/mg DM) (p = 0.01) and TP (2.33 vs. 2.52 mg GAE/mg DM) (p = 0.021).

Table 2. Secondary metabolites of *Paulownia shan tong* harvested in winter and spring.

Items	Winter	Spring	SEM	p Value
Total flavonoid (mg CE [1]/mg DM)	0.36 ^{b}	0.45 ^{a}	0.014	0.010
Total phenolic (mg GAE [2]/mg DM)	2.33 ^{b}	2.52 ^{a}	0.036	0.021
Condensed tannins (mg CE [1]/mg DM)	0.02	0.02	0.000	1.000
IC_{50}_DPPH (mg/mL)	0.32 ^{b}	0.22 ^{a}	0.008	0.009
IC_{50}_$ABTS^+$ (mg/mL)	0.41 ^{b}	0.31 ^{a}	0.005	0.002

Means in the same row with different superscripts differ, $p < 0.05$. SEM = Standard error of the mean. IC_{50} _DPPH (mg/mL); the inhibitory concentration of the sample needed to inhibit 50% of the DPPH radicals; IC_{50}_$ABTS^+$ (mg/mL): the inhibitory concentration of the sample needed to inhibit 50% of the ABTS-+ radicals; [1] CE = Catechin equivalents; [2] GAE = Gallic acid equivalents.

3.2. In Vitro Fermentation Kinetics

No animal species (rumen fluid donors) × harvest time interactions were observed for the fractional rate of fermentation, GP after 24 h of incubation, or average rate of GP parameters (Table 3). However, significant interactions ($p < 0.001$) were observed for the asymptotic GP (p = 0.041), lag time (p = 0.005), and half-time. The rumen fluid donor species and harvest times affected ($p < 0.05$) all measured parameters of kinetics of in vitro GP of *P. shan tong*. Concerning the rumen liquid source, higher values of A (112.8 vs. 98.4 mL/g DM) (p = 0.005), c (0.07 vs. 0.04 mL/h) ($p < 0.001$), G24 (79.6 vs. 49.2 mL/g DM) ($p < 0.001$), and average rate of GP (3.60 vs. 2.06 mL/h) ($p < 0.001$) were observed when *P. shan tong* leaves were incubated with the rumen liquid of goats compared to sheep. Conversely, the highest values for L (5.56 vs. 4.74 h) (p = 0.011) and half-time of GP (23.8 vs. 15.6 h) ($p < 0.001$) were associated with *P. shan tong* leaves incubated in sheep rumen fluid. The winter harvest of *P. shan tong* showed higher A (122.1 vs. 89.0 mL/g DM) ($p < 0.001$), G24 (0.04 vs. 0.06 mL/h) (p = 0.002), average rate of GP (3.03 vs. 2.62 mL/h) (p = 0.002), and half-time of GP (21.8 vs. 17.6 h) ($p < 0.001$) compared to the spring harvest, which showed higher L (5.82 4.47 h) (p = 0.001).

Table 3. Kinetics of in vitro gas production of *Paulownia shan tong* harvested in winter and spring and incubated with rumen liquor from goats or sheep.

	Animal Species		Harvest Season			p Value		
	Goat	Sheep	Winter	Spring	SEM	Animal	Harvest	Animal × Harvest
A (mL/g DM)	112.8 ^{a}	98.4 ^{b}	122.1 ^{a}	89.0 ^{b}	1.80	0.005	<0.001	0.041
c (per h)	0.07 ^{a}	0.04 ^{b}	0.04 ^{b}	0.06 ^{a}	0.002	<0.001	<0.001	0.408
Lag time (h)	4.74 ^{b}	5.56 ^{a}	4.47 ^{b}	5.82 ^{a}	0.177	0.011	0.001	0.005
G_{24} (mL/g DM)	79.6 ^{a}	49.2 ^{b}	68.8 ^{a}	60.1 ^{b}	1.4	<0.001	0.002	0.272
Average rate of gas production (mL/h)	3.60 ^{a}	2.06 ^{b}	3.03 ^{a}	2.62 ^{b}	0.064	<0.001	0.002	0.062
Half-time of gas production (h)	15.6 ^{b}	23.8 ^{a}	21.8 ^{a}	17.6 ^{b}	0.3	<0.001	<0.001	<0.001

Means in the same row (for animal or harvest) with different superscripts differ, $p < 0.05$. SEM = Standard error of the mean. A, asymptotic gas production (mL/g DM incubated); c, fractional rate of fermentation (/h); G_{24}, gas production after 24 h of incubation (mL/g DM incubated); average rate of gas production, (mL/h); half-time (h), time to reach a value of gas production equal to half of the asymptote.

No animal species (rumen liquor donors) × harvest time interactions were observed for the ME, dOM, UFL, UFV, PDIA, PDIN, and PDIE (Table 4). Higher ($p < 0.001$) ME (6.03 vs. 5.27 MJ/kg DM), dOM (45.1 vs. 39.7%), UFL (0.44 vs. 0.38 MJ/kg DM), UFV (0.33 vs. 0.26 MJ/kg DM), and PDIE (87.6 vs. 83.3 g/kg DM) concentrations were observed when the *P. shan tong* was incubated with rumen liquor from goats compared to that from sheep. Moreover, higher ($p < 0.05$) ME (5.72 vs. 5.59 MJ/kg DM), dOM (43.2 vs. 41.6%), UFL (0.42

vs. 0.40 MJ/kg DM), and UFV (0.31 vs. 0.28 MJ/kg DM) concentrations were observed in the winter harvest of *P. shan tong* compared to the spring harvest.

Table 4. In vitro organic matter digestibility, metabolizable energy for ruminants, and microbial protein supply of *Paulownia shan tong*.

	Animal Species		Harvest Season			*p* Value		
	Goat	**Sheep**	**Winter**	**Spring**	**SEM**	**Animal**	**Harvest**	**Animal × Harvest**
ME (MJ/kg DM)	6.03 [a]	5.27 [b]	5.72 [a]	5.59 [b]	0.035	<0.001	0.030	0.278
dOM (%)	45.1 [a]	39.7 [b]	43.2 [a]	41.6 [b]	0.25	<0.001	0.002	0.290
UFL (MJ/kg DM)	0.44 [a]	0.38 [b]	0.42 [a]	0.40 [b]	0.003	<0.001	0.001	0.305
UFV (MJ/kg DM)	0.33 [a]	0.26 [b]	0.31 [a]	0.28 [b]	0.003	<0.001	<0.001	0.191
PDIA (g/kg DM)	58.3	58.3	57.7	58.8	0.37	1.000	0.058	1.000
PDIN (g/kg DM)	124.8	124.8	123.7	126.0	0.93	1.000	0.113	1.000
PDIE (g/kg DM)	87.6 [a]	83.3 [b]	85.7 [a]	85.3 [b]	0.64	0.002	0.659	0.433

Means in the same row (for animal or harvest) with different superscripts differ, $p < 0.05$. SEM: standard error of the mean. dOM: Organic matter digestibility; ME: Metabolizable energy for ruminants; UFL: Forage unit for lactation; UFV: Forage unit for meat production; PDIA: Protein digestible in the small intestine supplied by rumen—undegraded dietary protein; PDIN: PDIA + PDI supplied by microbial protein formed from feed protein degraded in the rumen; PDIE: PDIA + PDI supplied by microbial protein from rumen—fermented organic matter.

3.3. Overall Multivariate Analysis

The cluster analysis using all chemical composition data (Figure 1) showed a significant discrimination between both seasons (winter vs. spring) when all data were used in a multivariate analysis. A second analysis was performed separately on the fermentation kinetic data (Figure 2). In this case, four clusters were identified (similarity > 97 %) clearly separating both seasons and showing significant differences between sheep and goats in their potential to digest *P. shan tong* leaves in the rumen.

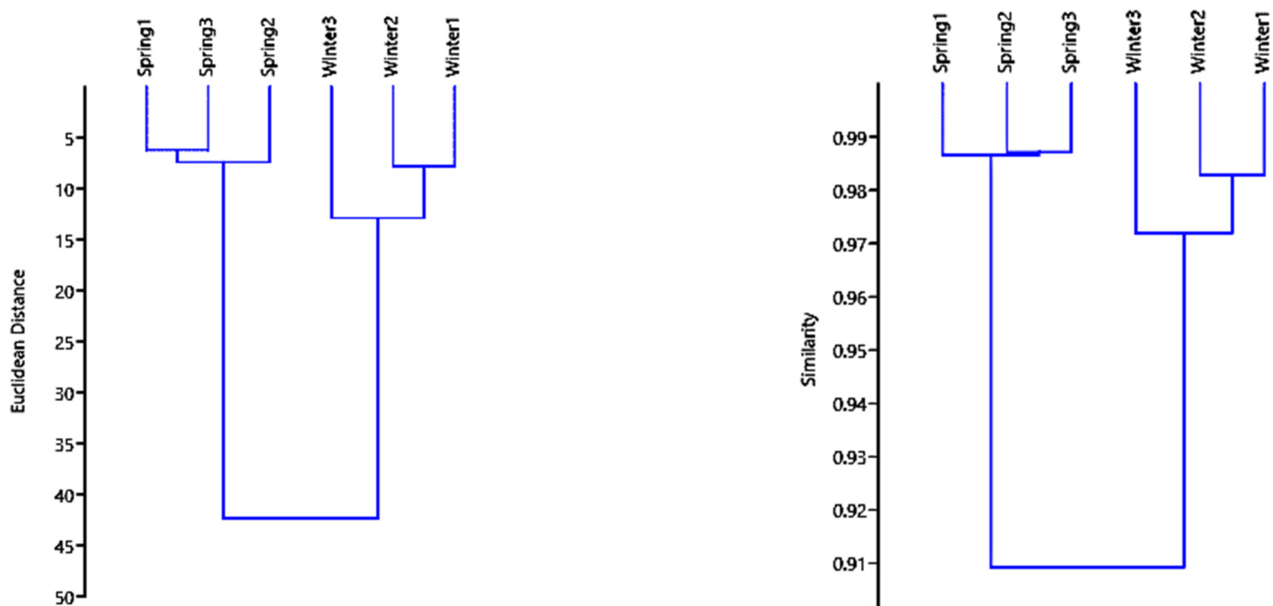

Figure 1. Hierarchical clustering of harvest seasons (spring vs. winter) using chemical composition and in vitro fermentation kinetic data of *Paulownia shan tong*.

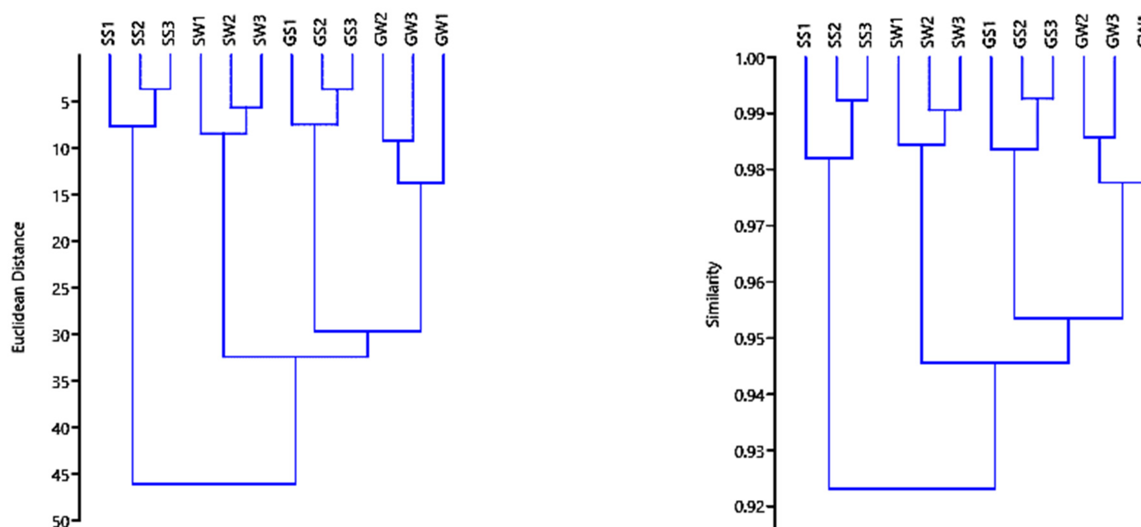

Figure 2. Hierarchical clustering of harvest seasons of *Paulownia shan tong* using in vitro fermentation kinetic data using sheep or goat ruminal inocula (SS = sheep—spring; SW = sheep—winter; GS = goat—spring; GW = goat—winter).

4. Discussion

4.1. Chemical Composition and Secondary Metabolites

The leaves of many trees contain high concentrations of basic nutrients and bioactive substances, making them recognized sources of feed for ruminants year-round [3,31]. One of these trees is *Paulownia*, planted mainly for wood production and producing about 54 tons of by-product per hectare [8]. In Tunisia, planting *Paulownia* is encouraged by the government, especially in areas affected by erosion and poor soil as well as in industrial zones. *Paulownia* is considered able to improve the soil and control the pollution of subsoil waters that have high levels of heavy metals, chemicals, and animal-waste pollutants. In addition to controlling erosion, *Paulownia* serves as a shelter and barrier from the sun and winds, purifies the air from pollutants, and releases large quantities of O_2. It also has the capacity to absorb CO_2 ten times more than other tree species and contributes to the enrichment of the landscape and the biological diversity of the country. It can also be cultivated with other plant species (intercropping). In our present study, *P. shong tong* was studied to evaluate its nutritional value for small ruminant species based on its chemical composition and in vitro fermentation. As mentioned before, sampling was carried out in two consecutive seasons (winter and spring). Leaves from the spring harvest showed higher concentrations of OM, lipids, NDF, ADF, and ADL (Table 1). Other studies carried out in the literature [17,32] pointed out the significant effect of the harvest time on the nutrient concentrations of plants. The environmental conditions and maturation state are the main factors that influence the chemical composition of plants [33,34]. The precipitation level determines the DM content in plants, especially in the initial phase of plant growth [32].

The mineral contents reported in our present study varied between 10.2% and 9.5% for the winter and spring harvest, respectively. Similar concentrations have been obtained by Bodnar et al. [6], with reported values of 10.5% and 10.8%, respectively. However, higher mineral contents (14.9%) were reported by Ganchev et al. [35]. This discrepancy could be attributed to variation in environmental conditions and the maturation state of the plant [33,34]. It is well documented that the concentration of CP in plants depends mainly on the harvest time, plant growth phase, fertilizer applied, and soil conditions [32,35]. However, the CP contents reported herein remained stable throughout the growing season and ranged in a very narrow interval, being 19.4% and 19.8% for the winter and spring harvests, respectively. In contrast to this observed evolution through the seasons, Ganchev et al. [35] observed that leaves of *P. shan tong* collected in spring had a higher CP concentration than leaves collected in the late autumn. As compared with our results, the literature

pointed out lower (17.4% [36]) and higher (22.6% [37] and (27.1%) [38]) CP contents in *Paulownia* species. These differences could be attributed partly to different climatic conditions, varieties studied, and harvesting time [32,35]. The CP of *P. shan tong* is considered moderate compared with leaves of *Moringa oleifera*, which reaches more than 27% CP [39], but similar to those recorded for alfalfa (17 to 19%) [40]. However, the CP reported in the present study is much higher than those measured in acacia species (13.8%) [35] and in some major cereals such as wheat (14.2%), rye (12.7%), barley (12.3%), and corn (10.7%), which are widely used in animal feed production [41]. Additionally, the CP of *P. shan tong* is above the minimum threshold (80 g/kg DM) required for rumen microbial growth and activity [42].

Regarding the fiber fraction concentration, the leaves of *P. shan tong* contained a high NDF concentration (55.6 and 69.7%) for the winter and spring harvests, respectively. These values are higher than those observed by Al-Sagheer et al. [36] (40.5%). These values are twice as high as the fiber concentration of *Ajuga iva* [2]. A high fiber concentration in plants has the potential to negatively affect ruminal fermentation and decrease the microflora activity [2].

The concentration of condensed tannins did not differ between harvests (Table 2). This would partially explain the unchanged CP concentration in the leaves. Tannins may partially inactivate the proteolytic enzymes by forming complexes with proteins [9].

Leaves obtained from the winter harvest showed higher IC_{50}_DPPH and IC_{50}_$ABTS^+$, which were used as indicators of the antioxidant activity (AA). Both the $ABTS^+$ and DPPH assays are widely used to assess the AA of natural products and show the radical-scavenging ability of antioxidants [43]. In the present experiment, the DPPH and $ABTS^+$ (0.32 and 0.41 mg/mL, respectively) were higher in the winter harvest compared to those for the spring harvest (0.22 and 0.31 mg/mL, respectively). Higher IC_{50} values indicate a lower AA [43]. Through the results, we can distinguish an increase in the percentage of inhibition according to the concentration of the two radicals. Generally, polyphenols with a high number of hydroxyl groups show a very high antioxidant activity [44]. Given that the extract of leaves from the spring harvest had a higher polyphenol content than that of leaves from the winter harvest (2.510.07 mg EAG/g DM), which had lower polyphenol contents (2.320.05 mg EAG/g DM), it would explain the findings of the present study. A high AA of leaves and flowers of *P. tomentosa* was confirmed by Uğuz and Kara [45].

Leaves from the spring harvest had higher concentrations of TF and TP, which may partially explain its high antioxidant activity [2,12]. Higher concentrations of these secondary compounds may exhibit antimicrobial activity, reduce methane emission, and change the ruminal fermentation pattern [12]. The concentrations of TF (0.36 and 0.45 mg CE/mg DM), TP (2.33 and2.52 mg GAE/mg DM), and TCT (0.02 mg CE/mg DM) for the winter and spring harvests, respectively, were favorable in the leaves as a feed for ruminants. Phenolics at high concentrations have a toxic effect on ruminal bacteria and protozoa [12]. However, no such effects were observed in this study, indicating that their levels were within the acceptable ranges of tolerance for rumen microbes. The review conducted by Kholif and Olafadehan [12] stated that low and sometimes medium levels of secondary metabolites in plants have positive effects on ruminal fermentation and productivity in vivo and in vitro compared to high levels.

To sum up, the concentrations of nutrients and bioactive compounds in the leaves indicated that *P. shan tong* leaves could be used as a dietary component for ruminants.

4.2. In Vitro Fermentation Kinetics

No interactions between animal species (rumen liquor donors) and harvest time (during winter or spring) were observed for the fractional rate of fermentation, GP after 24 h of incubation, average rate of GP parameters (Table 3), ME, dOM, UFL, UFV, PDIA, PDIN, or PDIE (Table 4). Therefore, only the main effects of species and season are discussed.

It is well-reported that source of rumen liquor and harvest times affected all measured parameters of kinetics of in vitro GP of *P. shan tong*, indicating different microbial profiles

between goats and sheep [16]. The ruminal microbial population depends mainly on the type of diet fed. In our study, sheep and goats, the donors of ruminal liquid, were fed differently. The sheep were raised in a feedlot; however, the goats were grazing in mountainous areas dominated by different tanniferous species. Therefore, the microbial population in the rumen liquid of both species would be expected to vary. Other factors can cause some variation in the inoculum, including host animal effects, sampling time and source, as well as sample preparation and inoculation [46]. Differences in dentition, chewing/eating behavior, gut physiology, compartment dimensions, and retention time influence gut microflora [47]. It is pertinent to mention that ruminants use a secondary defense mechanism present in their saliva against tanniferous species. In this context, Ammar et al. [48] revealed that pre-incubating tanniferous samples with saliva from sheep adapted to the ingestion of tannins increased their in vitro digestibility compared to pre-incubation in saliva of non-adapted sheep. In our present study, incubation of *P. shan tong* leaves with rumen liquor from goats increased the asymptotic GP, fractional rate of fermentation, GP after 24 h of incubation, and average rate of GP (Table 3), confirming the results of other experiments that goats are more efficient than sheep at digesting feedstuffs [47]. The obtained results are inconsistent with the results observed by others [47], who reported higher asymptotic GP and GP after 24 h of incubation when browse foliage was incubated in buffered sheep rumen liquor than goats. The concentrations of nutrients and phenolic compounds between *P. shan tong* and browse foliage in the study carried out by Ammar et al. [47] may be responsible for these results' inconsistency. Likewise, incubation of *P. shan tong* leaves with rumen liquor from goats increased the lag time and half-time of GP, revealing that the time taken for microbes to adhere to the substrates and microbial attachment to insoluble substrate was higher in goats compared to sheep [16]. Moreover, leaves of *P. shan tong* collected in winter showed higher A, G_{24}, average rate of GP, and half-time of GP (Table 3), indicating a higher fermentability compared to those collected in spring. The higher concentrations of NDF, ADF, and ADL in the spring harvest and higher OM, CP, and fats may explain such results [49,50]. The higher rate of GP indicates more rapidly fermentable components in the leaves from the winter harvest. Regarding the harvesting time and rumen liquid source, the lag times of leaves of *P. shan tong* collected in the spring harvest and incubated in sheep rumen liquid gave rise to the highest value. This indicates that the time required for cellulolytic microorganisms to adhere to and colonize the dietary fiber was shorter in goats than sheep [51]. This result may be explained by the richness of fiber and cellulosic walls, especially for those issued from the spring harvest. Gas production depends mainly on the degradability of soluble components in the incubated substrates and the partitioning of fermented substrates [52,53]. The fiber fractions' concentrations increased in the spring harvest compared to the winter harvest, which may explain the late produced GP [2].

Incubation of *P. shan tong* leaves with rumen liquor from goats showed higher ME, dOM, UFL, UFV, and PDIE concentrations (Table 4). The different effects of rumen inoculum on OM degradability and microbial protein may be due to different bacterial and protozoal populations and microbial activity in goats and sheep [16]. Almost all the experiments reported in the literature stated negligible differences between sheep and goats in rumen degradability for high-quality forages and roughages. However, it is believed that goats are used to digesting forages with higher fiber and lower N contents than sheep [54]. In the present experiment, the leaves of *P. shan tong* contained about 19 to 20% CP and 66 to 70% NDF, which may explain the observed superior results in goats than sheep. The higher dOM and estimated microbial protein production are good evidence of the higher counts of cellulolytic bacteria, higher enzyme activity to hydrolyze structural polysaccharides, and favorable and stable conditions in the rumen of goats. Our results indicate that goats were more efficient than sheep in digesting *P. shan tong* as a feedstuff. This could be related to the fact that goats are more tolerant to the presence of phenolic compounds and tannins in the leaves due to the presence of different bacterial species (*Streptococcus caprinus*) in their rumen as a possible adaptation to the ingestion of shrub species [55]. In this

context, it is worth remembering that the rumen fluid used in the current study for in vitro incubations was extracted from slaughtered animals raised in the northwest of Tunisia. In this area, goats are generally raised under an extensive production system, grazing in mountainous areas dominated by tanniferous species. However, in these same areas, fattening sheep are raised under the feedlot system, especially during the last fattening period, in order to obtain a higher average gain. Therefore, an adaptation of goats' rumen microflora occurred and translated into higher digestibility and fermentative capacity of goats compared to sheep.

With regards to the harvesting time, leaves of *P. shan tong* collected in winter have higher ME, dOM, UFL, and UFV concentrations than those obtained in spring (Table 4). This would indicate higher energy availability for milk and meat production for animals consuming the leaves from the winter harvest [30]. A higher dOM is most often associated with higher levels of nonstructural carbohydrates and CP, which contain less fiber and are easy to ferment [56]. Moreover, the measured parameters of protein value indicate that the spring harvest leaves had the lowest nutritive value of protein compared to those of the winter harvest [30]. Greater concentrations of protein undegraded in the rumen but truly digestible in the small intestine, as well as true protein absorbable in the small intestine when rumen fermentable energy is limiting, are good indicators of high nutritive value. Lower degradability at the beginning of incubation indicates greater bypass protein that will be utilized in the duodenum [57]. Microorganisms could easily attack more-degradable protein in the rumen and reflect greater protein solubility [5].

5. Conclusions

These preliminary results show that *P. shan tong* leaves are a good source of protein, lipids, fibers, and minerals with moderate amounts of phenolic substances. The nutritive value of the leaves harvested in winter was higher than those harvested in spring. The harvest of *P. shan tong* during winter showed a higher gas production and rate of gas production compared to the harvest during spring. The fermentability of the leaves was higher when incubated with goats' rumen fluid than with sheep. Higher values and rate of gas production were observed when *P. shan tong* leaves were incubated with the rumen liquid of goats as compared to incubation in sheep rumen liquid. However, a higher lag of gas production and half-time of gas production were associated with *P. shan tong* leaves incubated in sheep rumen fluid. The results indicate that the leaves of *P. shan tong* may be considered as an unconventional feedstuff for goat-based production systems. The in vitro results should be validated under in vivo conditions, as the bioactive compounds present in *P. shan tong* leaves could interact with the rumen microbiota in a more multidirectional manner. Moreover, the incorporation rate of *P. shan tong* leaves in sheep and goats nutrition should be defined for each ruminant species. One of our study's limitations was the inclusion of only one year of data. Future studies should consider including data from multiple years and under different environmental conditions.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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