

Article

Fermentation Kinetics and Digestibility of Cultivated Mediterranean Leguminous Shrubs: Effects of Season, Plant Part and Irrigation

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Abstract

This study evaluates the effects of plant part (leaves vs. stems), harvest season (spring vs. autumn), and irrigation on the chemical composition, in vitro ruminal fermentation kinetics, and digestibility of three Mediterranean leguminous shrubs: *Acacia fimbriata*, *Medicago arborea* and *Anthyllis barba-jovis*. Samples were collected under field conditions, analysed for chemical composition, and assessed for in vitro ruminal fermentation, including incubations with and without polyethylene glycol (PEG) to assess the biological activity of phenolic compounds. Crude protein and fibre fractions did not differ significantly among species; ash was highest ($p < 0.05$) in *Ac. fimbriata*. In vitro fermentation kinetics were similar among species; however, the response to PEG was higher ($p < 0.05$) for *Ac. fimbriata* than for *M. arborea* and *An. barba-jovis*. In vitro digestibility was superior ($p < 0.05$) in *M. arborea* and *An. barba-jovis*. Leaves contained higher ash and protein contents and less fibre than stems, resulting in greater gas production, fermentation rate, and digestibility ($p < 0.05$). Spring-harvested fodder showed higher digestibility than autumn samples. Irrigation had negligible effects, except for a marginal numerical but nonsignificant ($p = 0.05$) greater extent of degradation observed in browse prior to irrigation. Overall, *M. arborea* showed a better nutritional quality, whereas *Ac. fimbriata* was the species with the lowest nutritive value. Forage quality was strongly influenced by plant part and harvest season, with leaves and fodder in spring showing superior potential as a feedstuff for ruminants.



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1. Introduction

Ruminant livestock production systems, particularly sheep and goat farming, frequently encounter productivity bottlenecks due to pronounced seasonal variability, recurrent drought, and the inherently low yield and quality of natural pastures in arid and semi-arid environments. These constraints result in periodic shortages of suitable-quality forage, especially during the dry season when herbaceous vegetation becomes scarce and highly lignified, ultimately limiting animal performance in terms of growth, reproduction, or milk production. To face these challenges, the strategic incorporation of agro-industrial by-products into ruminant diets [1], alongside the utilisation of drought-resilient shrubs and fodder trees through grazing or browsing [2–4], has been proposed as key adaptive strategies for sustaining small-ruminant production in climate-vulnerable regions. The inherent nutritional value of such feedstuffs is a primary determinant of their suitability to be incorporated in ruminant diets and their subsequent impact on animal performance. Generally, forages characterised by high crude protein content and low fibre fractions exhibit superior digestibility, thereby resulting in a more efficient supply of energy and essential nutrients [5]. However, the presence of secondary plant metabolites (such as tannins) may pose a significant concern because these compounds can impair nutrient utilisation by inhibiting ruminal microbial fermentation [6–8]. Therefore, identifying plant species with lower tannin concentrations or implementing interventions to enhance nutrient bioavailability, such as the application of polyethylene glycol (PEG) to neutralise tannin-binding effects, remains a focal point of contemporary ruminant nutrition research [9].

Shrub species indigenous to Mediterranean ecosystems represent a strategic and sustainable forage reserve capable of filling seasonal feed gaps, particularly during the summer months when herbaceous vegetation becomes senescent. Within this context, Fabaceae species (such as *Acacia* spp., *Medicago* spp., or *Anthyllis* spp.) are of particular interest due to their robust adaptability to arid conditions, deep rooting systems, nitrogen-fixing ability, and persistence under limited rainfall [10,11]. Frequently, these leguminous plants contain tannin compounds that may offer secondary benefits, such as anthelmintic effects and the protection of feed protein against ruminal degradation, thus increasing the flow of bypass protein [12]. However, these phenolic compounds may also hinder the efficiency of ruminal digestion [13]. Although PEG has been validated as an effective agent for binding tannins and protecting microbial activity [6,14,15], quantitative information linking tannin activity to actual fermentation responses in many Mediterranean browse species remains limited. Consequently, comprehensive data regarding the nutritional profile and digestibility of legume shrub/tree species from arid or semiarid regions remain sparse. Specifically, there is a knowledge gap concerning how their nutritive value fluctuates in response to external variables such as plant morphology, phenological stage, and water availability.

Drought-induced water stress further impacts forage yield and quality because of its influence on plant metabolism, structural development, and nutrient allocation. Irrigation is known to modulate nutrient uptake, dry matter yield, and the expression of secondary metabolites in various forage crops [16,17]. However, evidence describing whether improved water availability enhances nutritive value or merely increases biomass production in perennial shrubs is still inconclusive, and comparative data examining the influence of irrigation on the digestibility of leguminous tree fodder are limited, yet necessary for the development of sustainable livestock systems. Furthermore, the partitioning of nutrients is

highly variable across plant tissues and seasons. Leaves generally have higher protein and lower fibre compared to stems [18], and thus ruminal degradation of stems is more limited due to their high lignocellulosic content [5,19]. Seasonal changes also affect quality so that spring-harvested forages have higher protein and less lignified structural carbohydrates than those harvested in autumn due to differences in plant maturity and lignification processes [18,20]. In Mediterranean silvopastoral systems, the integration of fodder species into small-ruminant feeding is rarely managed as a single, uniform resource; instead, practical utilisation must account for the seasonal availability of feed, the partitioning of forage quality between leaf and stem fractions accessible to the browsing animal, and the degree to which tannins modify nutrient availability in the rumen [7,21]. Matching shrub utilisation to seasonal windows of superior leaf quality and targeted leaf-fraction harvesting are therefore key management decisions with direct consequences for animal performance during critical production periods such as lactation and late gestation. Despite this, the evidence base needed to guide such decisions for several promising Mediterranean leguminous shrubs and fodder trees remains incomplete. Despite recognition of individual effects, integrated evaluation of species differences, plant fraction, seasonal dynamics, irrigation regime, and the functional role of tannins in modulating rumen fermentation remains scarce. The study reported herein investigates the influence of species, botanical plant part, seasonality, and irrigation on the chemical composition, gas production kinetics, and in vitro digestibility of cultivated *Acacia fimbriata*, *Medicago arborea*, and *Anthyllis barba-jovis*. These three species were selected because, despite their recognised adaptability to Mediterranean drylands, available information on their nutritive value remains fragmentary and, in some respects, contradictory. *M. arborea* is comparatively well characterised as a Mediterranean fodder shrub, yet most existing reports describe its nutritive value under a single harvest or utilisation condition rather than across combined seasonal and irrigation regimes [18,22,23]. Data on *Ac. fimbriata* and *An. barba-jovis* are even more sparsely documented, even though related congeneric species have been studied elsewhere [24–28]. Consequently, neither the comparative nutritive ranking of these three particular species nor their response to combined seasonal and water-availability gradients has been previously established within an integrated experimental framework. This evidence gap, together with the practical need to align shrub utilisation with seasonal feed shortages and the leaf-to-stem partitioning of fodder quality that characterises Mediterranean silvopastoral systems [29–31], motivated the present comparative approach integrating plant species, plant part, season and irrigation as potential influential factors, supplemented by a PEG bioassay to investigate the contribution of biologically active tannins.

Accordingly, this study tested the hypotheses that (i) nutritive value and tannin bioactivity differ among the three species; (ii) leaves are more digestible than stems across all species; (iii) the nutritive value of tree foliage differs depending on the period in which it is utilised as a browse resource by small ruminants; and (iv) irrigation influences the composition and digestibility of this foliage. The specific objectives were to quantify the chemical composition, in vitro gas production kinetics, digestibility, and PEG-responsive tannin activity of *Ac. fimbriata*, *M. arborea*, and *An. barba-jovis*, and to determine the independent contributions of plant part, harvest season, and irrigation status to these traits, thereby identifying the combinations of species, part, and season that maximise feeding value for small ruminants in Mediterranean drylands.

2. Materials and Methods

2.1. Plant Sampling

The three leguminous shrub species investigated were *Acacia fimbriata* A.Cunn. ex G.Don, *Medicago arborea* L., and *Anthyllis barba-jovis* L.

In 2005, various tree and forb seedlings were obtained from the nursery stock of the Tunisian Ministry of Agriculture and then established at the Ecole Supérieure d'Agriculture de Mograne (Mograne-Zaghouan, Tunisia, 10°46' E, 36°26' N; 156 m altitude). The plantation was intended to serve both as a windbreak and as a supplementary source of fodder for small ruminants. Seedlings were planted in rows spaced 1.5 m apart, with four rows per species and approximately 1 m between plants within each row. During the establishment phase (years 1–3), it was irrigated twice weekly from May to September and once weekly from October to April. The experimental plot was protected from herbivory, and competing weeds were manually controlled around the plant bases. No pesticide applications were required during either the establishment or growth periods. Growth was supported by annual fertilisation with locally sourced livestock manure. Climatic data for the study period are summarised in Table 1.

Table 1. Monthly temperature and precipitation recorded at the Meteorological Station at the Ecole Supérieure d'Agriculture de Mograne (Tunisia).

Month	Average Years 2001–2007			Year 2008	
	Temperature (°C) Minimum	Temperature (°C) Maximum	Average Rainfall (mm/Month)	Average Temperature (°C)	Rainfall (mm)/Month
January	5.4	12.4	103.0	11.1	143.8
February	5.5	16.1	45.0	10.3	56.1
March	7.4	20.0	31.8	12.9	42.3
April	10.1	19.6	37.6	15.0	181.1
May	13.7	26.0	34.0	19.8	12.1
June	16.4	34.8	16.0	25.3	12.3
July	19.7	37.0	5.33	26.7	5.3
August	20.0	36.9	12.0	26.9	15.1
September	18.0	31.6	80.8	25.0	37.4
October	15.2	29.4	45.8	20.9	14.2
November	10.1	21.6	42.6	15.3	17.3
December	6.2	16.9	34.3	11.0	9.4

As shown in Table 1, average maximum temperatures in the study area rose from approximately 20 °C in March to a summer peak above 36 °C in June–August, while rainfall was concentrated mainly between October and April. This pattern of high summer temperatures and minimal rainfall during the months preceding the autumn sampling provides the climatic basis for the more advanced phenological stage and greater lignification observed at that time, relative to the actively growing spring condition. By 2008 (fourth year of growth, after adaptation to local conditions), the plants exceeded 3 m in height and were irrigated only occasionally. Sampling was conducted in spring and autumn 2008 and, within each season, before and after irrigation. The first samples (pre-watering) were collected on 12 May, and after sampling the plants were irrigated. Post-irrigation samples were collected on 14 May. Water was applied manually using a rubber hose to the base of the plants until the furrows were filled (soil saturation by surface immersion), ensuring uniform distribution across the root zones of the established shrubs. After that, plants were not irrigated again until the autumn samplings, in November (23 and 27), when the same protocol (pre-watering sampling, irrigation and post-irrigation sampling) was repeated. During the spring sampling, all three species were in a stage of active vegetative growth, characterised by newly flushed leaves and elongating terminal shoots. Conversely, when sampled in autumn, prior to the onset of winter dormancy, the plants had reached a more advanced phenological stage. This later phase was characterised by leaf hardening and

partial lignification of the shoots, consistent with the post-summer-drought status typical of these species in the Mediterranean basin.

Six healthy, mature individuals of each species were selected as representative specimens at the study site. The trunks of these plants were labelled with permanent markings to ensure consistent longitudinal sampling. The same individuals were sampled across two seasons; within each season, samples were collected immediately before and after irrigation. At each sampling event, terminal branches containing green leaves and young shoots (fine twigs) were manually harvested using pruning shears from the lower, middle, and upper canopy of each plant. The biomass harvested from the six plants at each collection time was pooled pairwise, yielding three composite samples per species. Each pair of plants served as a sampling unit to provide three biological replicates ($n = 3$) per species, whereas season and irrigation timing represented repeated-measures factors on the same sampling units. Separate fractions of leaves and current-season twigs were isolated from every composite sample. Leaves and stems (fine twigs) were separated immediately after collection by hand-stripping the leaves from each harvested shoot, retaining only the woody axis as the stem fraction; petioles were included in the leaf fraction. This manual separation protocol was adopted because mechanical processing methods are poorly suited to the small, brittle leaflets of these species and introduce a risk of cross-contamination between tissue types. Leaves and stems were analysed as separate fractions rather than as whole-shoot material. Characterising each fraction independently allows the relative contribution of leaf-harvesting intensity to overall forage quality to be estimated for any leaf-to-stem ratio encountered in the field, an assessment that is precluded when analysing only bulked, whole-shoot samples.

The resulting material was air-dried at room temperature (20–25 °C) under shade for 5–7 days until constant weight and ground (through a 1 mm screen) for subsequent analysis. Ground samples were stored in sealed polyethylene bags at room temperature, protected from light and moisture, until chemical and *in vitro* assays were conducted in the laboratories of the Instituto de Ganadería de Montaña (CSIC-Universidad de León, Spain) in 2010. Ground plant material of this type is chemically stable when kept sealed, dry, and protected from light, and the chemical composition and digestibility data reported here are not expected to be affected by storage duration, as the controlled storage conditions ensured the compositional stability of the plant material prior to analysis.

2.2. Chemical Analysis

The chemical composition of the collected browse material was analysed in the laboratory on dried samples. Feed samples were analysed for dry matter (DM, ISO 6496: [32]), ash (ISO 5984: [33]), and crude protein (CP, ISO 5983-2: [34]). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin (ADL) were determined as described by Van Soest et al. [35], using a fibre analyser (Ankom Technology Corp., Macedon, NY, USA). Neutral detergent fibre was assayed with sodium sulphite and α -amylase and expressed with residual ash.

2.3. *In Vitro* Digestibility

In vitro digestibility was determined following the method of Goering and Van Soest [36] with the modifications described by Ammar et al. [19], using a DAISYII incubator, digestion jars, and the ANKOM filter bag technology (ANKOM, Macedon, NY, USA). The experimental protocols were approved (AGL 2010-19094) by the Institutional Ethics Committee of the Instituto de Ganadería de Montaña (CSIC-Universidad de León). The use of only *in vitro* techniques is consistent with the 3Rs principles of Replacement and Reduction, as no live animal experimentation was required. Collection and preparation of rumen fluid were as

described by Vargas et al. [37]. Samples of rumen contents from sheep were collected from a commercial abattoir. Strict criteria were applied to the selection of animals to collect rumen digesta, controlling for age (only adult sheep) and origin (farms where sheep were fed mainly by grazing or forage-based diets). When animals were slaughtered and eviscerated, rumina were opened and emptied, collecting well-mixed samples of rumen contents in thermos flasks that were immediately carried to the laboratory. The time from collection to the culture inoculation was less than one hour. All rumen digesta collected from different sheep was pooled and strained through four layers of cheesecloth before dilution with buffer. To determine the *in vitro* DM digestibility, the bags containing the samples were incubated in diluted rumen fluid at 39 °C under CO₂ for 48 h following the procedures described in detail by Horst et al. [38]. After incubation, bags were rinsed thoroughly under tap cold water and then oven-dried at 55 °C for 48 h. Dried bags were weighed to determine the remaining residue in the bags to calculate the apparent DM disappearance at 48 h (D48_{DM}). Afterward, bags were washed with a neutral detergent at 100 °C for 1 h and then oven-dried at 55 °C for 48 h. The residue remaining after neutral detergent extraction was assumed to be the truly undigested DM and was used to calculate the true *in vitro* DM digestibility (IVDMD) [36]. The same procedures (ANKOM filter bag technology) were followed with incubation in diluted rumen extended to 144 h. The residue remaining after that long incubation time, rinsing in cold water, and oven-drying was used to calculate the apparent DM disappearance at 144 h (D144_{DM}). After neutral detergent extraction and oven-drying, the weight of the residue was used to estimate the potentially true DM degradability (PTDMD). For each incubation run, duplicate bottles were used per substrate sample. Incubations were repeated across three consecutive weeks.

2.4. *In Vitro* Fermentation Kinetics

Rumen fermentation kinetics of each browse sample were evaluated by using the *in vitro* gas production technique proposed by Theodorou et al. [39]. Dried and ground samples (500 mg) were incubated in sealed culture bottles containing 50 mL of diluted rumen fluid at 39 °C in a saturated CO₂ headspace. The rumen fluid was diluted 1:2 (*v/v*) with anaerobic buffer medium prepared as described by Theodorou et al. [39], containing macromineral, micromineral, buffer, and reducing solutions together with resazurin as a redox indicator; the resulting pH of the buffered rumen fluid was 6.8–6.9. Bottles were sealed with rubber stoppers and aluminium caps. In each run (three runs), two bottles per sample and four bottles without any substrate (blanks) were incubated. Gas production (GP) was measured using a pressure transducer at 3, 6, 9, 12, 16, 20, 24, 30, 36, 48, 60, 72, 96, 120, and 144 h of incubation [39]. Three incubation runs were conducted in three consecutive weeks.

The exponential model derived by France et al. [40] was fitted: $GP = A \left[1 - e^{-c(t-L)} \right]$, where *GP* (mL/g DM) represents cumulative gas production at time *t* (h), *A* (mL/g DM) is the asymptotic gas production, *c* (h^{−1}) is the fractional fermentation rate, and *L* (h) is the lag time. The model was fitted to the cumulative gas production profile of each individual bottle by nonlinear least-squares regression using the NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The volume of gas at 24 h of incubation (*G*₂₄, mL/g DM) and the average fermentation rate were calculated. The extent of degradation in the rumen (g DM degraded/kg DM ingested) for a given rate of passage (*k*, h^{−1}) was estimated following the approach derived by France et al. [40]. A mean retention time of digesta in the rumen of 30 h (representative of sheep fed forage diets at maintenance level) was assumed, corresponding to a rate of passage (*k*) of 0.033 h^{−1}.

2.5. Tannin Biological Activity

Browse samples (leaves and twigs of the three species) were incubated in vitro in Wheaton bottles for the assessment of tannin biological activity. Each sample (300 mg) was incubated for either 6 or 24 h in 50 mL of buffered rumen fluid either with or without the addition of 500 mg of polyethylene glycol (PEG) at 39 °C and flushed with CO₂ [41]. The volume of gas produced after each incubation time (6 or 24 h) was measured. When a tannin-rich sample was incubated in the absence of PEG, microbial fermentation was predictably constrained by the presence of active tannins. Conversely, in parallel incubations of the same sample supplemented with PEG, the tannin-binding effects were neutralised. This counteracted the restriction on microbial activity and consequently increased the volume of gas released, adhering to the established physiological principles for polyphenol-rich browse and shrub species [4,41]. Consequently, biological tannin activity was assessed as the percentage increase in cumulative gas production resulting from PEG supplementation, expressed relative to the corresponding non-supplemented control as $[(\text{gas with PEG} - \text{gas without PEG}) \times 100] / \text{gas without PEG}$ [4].

2.6. Statistical Analysis

To evaluate the significance of the sources of variation affecting the nutritive value of the browse samples, data were subjected to analysis of variance using a linear fixed-effects model (PROC GLM, SAS version 9.4, SAS Institute Inc., Cary, NC, USA). For statistical analysis, the in vitro digestibility and gas production values were first averaged across the duplicate bottles within each run, and subsequently across the three independent incubation runs. Consequently, the statistical replication ($n = 3$) was strictly determined by the biological sampling units, corresponding to the three distinct pairs of field specimens sampled across seasons and irrigation events. Normality of residuals was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene’s test. Both assumptions were adequately met for all variables on the original scale, so no transformation was applied for any of the variables analysed. Data were initially evaluated using a factorial experimental design, with a full model that included all main effects and their two-way interactions. Given that all interaction terms were found to be non-significant (at $p > 0.10$) during that preliminary analysis (see Appendix A, Table A1), all these terms were pooled into the residual error term to improve the model parsimony and increase the sensitivity and statistical power of the tests for main effects. Thus, the final statistical model was simplified including only the main effects of species, plant part, season, and irrigation, in the form: $Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \varepsilon_{ijklm}$, where Y_{ijklm} is the individual observation for the m^{th} replicate of the i^{th} species, j^{th} plant part, k^{th} season, and l^{th} irrigation status, μ is the overall mean, α_i is the effect of the species ($i = 1, 2, 3$ for *Ac. fimbriata*, *M. arborea* and *An. barba-jovis*), β_j is the effect of the plant part ($j = 1, 2$ for leaves and twigs), γ_k is the effect of the season ($k = 1, 2$ for spring and autumn), δ_l is the effect of irrigation ($l = 1, 2$ for before and after irrigation), and ε_{ijklm} is the residual random error, which includes the accumulated variance associated with all non-significant interactions.

3. Results

Because no statistically significant two-way interactions were detected among species, season, irrigation, and plant part (Appendix A, Table A1), only the main effects of each factor are reported and discussed independently, except for specific findings regarding tannin biological activity. The results corresponding to each studied factor are presented in Tables 2–5.

3.1. Effects of Cultivated Species

Differences in chemical composition, in vitro gas production kinetics, and digestibility among the three leguminous shrub species are presented in Table 2.

Table 2. Effect of shrub species (*Acacia fimbriata*, *Medicago arborea* and *Anthyllis barba-jovis*) on chemical composition, in vitro gas production kinetics and in vitro digestibility.

	<i>Acacia fimbriata</i>	<i>Medicago arborea</i>	<i>Anthyllis barba-jovis</i>	SEM	<i>p</i> -Value
Chemical composition (g/kg DM)					
Ash	89 ^a	70 ^{ab}	57 ^b	8.4	0.046
Crude protein	122	150	138	11.2	0.237
Neutral detergent fibre	462	525	572	33.5	0.094
Acid detergent fibre	345	372	412	25.2	0.204
Lignin	118	116	133	8.1	0.309
Gas production kinetics					
A (mL/g DM)	101	137	117	11.3	0.113
c (per h)	0.066	0.059	0.056	0.0090	0.711
L (h)	0.18	0.68	0.40	0.182	0.177
Average rate (mL/h)	3.92	5.49	4.44	0.515	0.119
G ₂₄ (mL/g DM)	71.2	101.0	83.8	9.16	0.096
PEG ₆ (%)	16.69 ^a	0.21 ^b	5.19 ^b	1.62	<0.001
PEG ₂₄ (%)	15.37 ^a	0.16 ^b	4.99 ^b	1.66	<0.001
Extent of degradation in the rumen (g/kg DM)	484	516	505	16.0	0.386
In vitro digestibility (g/kg DM)					
D48 _{DM}	339 ^b	537 ^a	510 ^{ab}	48.0	0.019
IVDMD	532 ^b	632 ^{ab}	654 ^a	29.5	0.021
D144 _{DM}	389 ^b	596 ^a	567 ^{ab}	54.4	0.031
PTDMD	784	837	827	16.1	0.069

Values are means. SEM: standard error of the mean. A: asymptotic gas production; c: fractional rate of gas production; L: lag time; G₂₄: gas production at 24 h of incubation; PEG₆ and PEG₂₄: gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation; D48_{DM}: apparent in vitro digestibility; IVDMD: true in vitro digestibility after NDF treatment; D144_{DM}: dry matter disappearance after 144 h; PTDMD: potentially true DM digestibility. Within a row, means with different superscript letters differ significantly ($p < 0.05$).

Regarding chemical composition, *Ac. fimbriata* showed the highest and *An. barba-jovis* the lowest ash content ($p < 0.05$). Crude protein (CP) and fibre fractions (NDF, ADF, and ADL) were not significantly ($p > 0.05$) different among species. No significant differences ($p > 0.05$) were observed among species for the main parameters of in vitro gas production, including asymptotic gas production (A), rate constant (c), lag time (L), gas production at 24 h (G₂₄), average fermentation rate, and extent of degradation. In contrast, gas production measured in the presence of PEG was markedly affected by shrub species. Both PEG₆ and PEG₂₄ (gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation, respectively) values were significantly higher ($p < 0.05$) for *Ac. fimbriata* compared with *M. arborea* and *An. barba-jovis*, which showed similar values. The evaluation of tannin biological activity revealed distinct, species-specific responses across seasons, plant fractions, and irrigation regimes, as evidenced by the significant two-way interactions (Table A1). These effects will be described in the corresponding sections below. Significant differences were also detected for in vitro digestibility coefficients. D48_{DM}, IVDMD, and D144_{DM} were all significantly influenced by plant species ($p < 0.05$), though the specific patterns of variation differed among them. Both D48_{DM} and D144_{DM} were lower ($p < 0.05$) in *Ac. fimbriata* than in *M. arborea*, with *An. barba-jovis* exhibiting intermediate values. However, for IVDMD, the significant difference ($p < 0.05$) occurred between *Ac. fimbriata*

and *An. barba-jovis*, whereas *M. arborea* presented intermediate values. No significant differences were observed among species for PTDM. The species with the highest PEG response (*Ac. fimbriata*) was also the least digestible, consistent with an inhibitory effect of biologically active tannins on fermentation that was at least partly reversed by PEG.

3.2. Effects of Plant Part

Leaves showed significantly higher ash and CP contents than stems ($p < 0.05$), whereas stems contained greater ($p < 0.05$) concentrations of NDF, ADF, and ADL (Table 3). There were significant differences between leaves and stems in all parameters characterising in vitro gas production kinetics. Asymptotic gas production (A), lag time (L), gas production at 24 h (G_{24}), average fermentation rate, and extent of degradation were greater ($p < 0.05$) in leaves than in stems. In contrast, the fractional fermentation rate (parameter c) was higher ($p < 0.05$) in stems than in leaves. Gas production measured in the presence of polyethylene glycol was also influenced by the botanical part. Both PEG₆ and PEG₂₄ values were significantly higher ($p < 0.05$) for leaves than for stems. However, there was a significant ($p < 0.05$; Table A1) plant part $\times$ species interaction on tannin activity indices (PEG₆ or PEG₂₄). In *Ac. fimbriata*, tannin activity in terms of PEG₆ was similar in leaves (16.8%) and stems (PEG₆ = 16.6%). Conversely, in *M. arborea* (lowest tannin activity) and in *An. barba-jovis*, PEG₆ values were noticeably greater in leaves (5.4 and 7.9%, respectively) than in stems (not detected in *M. arborea* and 2.5% in *An. barba-jovis*). Significant ($p < 0.05$) differences were observed in in vitro digestibility parameters, as D48_{DM}, IVDMD, D144_{DM}, and PTDM were all significantly higher in leaves compared with stems. This pattern is consistent with the higher lignin concentration of stems, which corresponded to their lower digestibility across all four digestibility indices.

Table 3. Effect of botanical part (leaves vs. stems) on chemical composition, in vitro gas production kinetics and in vitro digestibility.

	Leaves	Stems	SEM	<i>p</i> -Value
Chemical composition (g/kg DM)				
Ash	91 ^a	54 ^b	6.8	0.001
Crude protein	191 ^a	82 ^b	9.2	<0.001
Neutral detergent fibre	393 ^b	646 ^a	27.4	<0.001
Acid detergent fibre	265 ^b	487 ^a	20.6	<0.001
Lignin	101 ^b	144 ^a	6.6	<0.001
Gas production kinetics				
A (mL/g DM)	144 ^a	93 ^b	9.2	<0.001
c (per h)	0.055 ^a	0.066 ^b	0.0074	0.031
L (h)	0.66 ^a	0.17 ^b	0.149	0.031
Average rate (mL/h)	5.38 ^a	3.85 ^b	0.420	0.019
G_{24} (mL/g DM)	103.3 ^a	67.3 ^b	7.48	0.003
PEG ₆ (%)	10.0 ^a	4.7 ^b	1.32	0.011
PEG ₂₄ (%)	9.1 ^a	4.6 ^b	1.36	0.031
Extent of degradation in the rumen (g/kg DM)	535 ^a	468 ^b	13.1	0.002
In vitro digestibility (g/kg DM)				
D48 _{DM}	584 ^a	340 ^b	39.2	<0.001
IVDMD	751 ^a	462 ^b	24.1	<0.001
D144 _{DM}	658 ^a	377 ^b	44.4	<0.001
PTDM	886 ^a	746 ^b	13.1	<0.001

Values are means. SEM: standard error of the mean. A : asymptotic gas production; c : fractional rate of gas production; L : lag time; G_{24} : gas production at 24 h of incubation; PEG₆ and PEG₂₄: gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation; D48_{DM}: apparent in vitro digestibility; IVDMD: true in vitro digestibility after NDF treatment; D144_{DM}: dry matter disappearance after 144 h; PTDM: potentially true DM digestibility. Within a row, means with different superscript letters differ significantly ($p < 0.05$).

3.3. Effects of Season

Season significantly affected the fibre composition of the forages (Table 4). NDF, ADF, and ADL contents were higher ($p < 0.05$) in autumn compared with spring, whereas ash and CP contents were not significantly influenced by season ($p > 0.05$). Some fermentation kinetics parameters were affected by season (Table 4). Asymptotic gas production (A) and average fermentation rate were higher ($p < 0.05$) in spring than in autumn. No significant seasonal differences ($p > 0.05$) were observed for the rate constant (c), lag time (L), G_{24} , or extent of degradation. Increase in gas production upon the addition of polyethylene glycol was not influenced by season, as no significant differences ($p > 0.05$) were detected for PEG₆ or PEG₂₄ between spring and autumn. However, there was a significant ($p < 0.05$; Table A1) season $\times$ species interaction on tannin activity indices (PEG₆ or PEG₂₄). In *Ac. fimbriata*, which exhibited the highest levels overall, a seasonal increase was observed, rising from spring (PEG₆ = 15.8%) to autumn (PEG₆ = 17.5%). A similar pattern was detected for *An. barba-jovis*, where PEG₆ rose from 4.1% in spring to 6.3% in autumn. Conversely, in *M. arborea* (lowest tannin activity), PEG₆ decreased from 3.0% in spring to negligible (not detected) values in autumn. Regarding in vitro digestibility, true digestibility (IVDMD) was significantly higher ($p < 0.05$) in spring than in autumn. However, D48_{DM}, D144_{DM}, and PTDM were not significantly affected by season.

Table 4. Effect of season (spring vs. autumn) on chemical composition, in vitro gas production kinetics and in vitro digestibility.

	Spring	Autumn	SEM	<i>p</i> -Value
Chemical composition (g/kg DM)				
Ash	79	66	6.8	0.196
Crude protein	141	132	9.2	0.481
Neutral detergent fibre	469 ^b	570 ^a	27.4	0.017
Acid detergent fibre	334 ^b	418 ^a	20.6	0.010
Lignin	111 ^b	134 ^a	6.6	0.023
Gas production kinetics				
A (mL/g DM)	133 ^a	103 ^b	9.2	0.034
c (per h)	0.064	0.057	0.0074	0.509
L (h)	0.54	0.29	0.149	0.256
Average rate (mL/h)	5.25 ^a	3.98 ^b	0.420	0.047
G_{24} (mL/g DM)	96.4	74.3	7.48	0.052
PEG ₆ (%)	7.55	7.18	1.32	0.847
PEG ₂₄ (%)	7.60	6.08	1.36	0.436
Extent of degradation in the rumen (g/kg DM)	508	495	13.1	0.496
In vitro digestibility (g/kg DM)				
D48 _{DM}	507	417	39.2	0.118
IVDMD	651 ^a	562 ^b	24.1	0.017
D144 _{DM}	529	506	44.4	0.725
PTDM	828	804	13.1	0.214

Values are means. SEM: standard error of the mean. A : asymptotic gas production; c : fractional rate of gas production; L : lag time; G_{24} : gas production at 24 h of incubation; PEG₆ and PEG₂₄: gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation; D48_{DM}: apparent in vitro digestibility; IVDMD: true in vitro digestibility after NDF treatment; D144_{DM}: dry matter disappearance after 144 h; PTDM: potentially true DM digestibility. Within a row, means with different superscript letters differ significantly ($p < 0.05$).

3.4. Effects of Irrigation

Irrigation did not significantly affect the chemical composition of the forages (Table 5). Similarly, most parameters describing in vitro gas production were not influenced by irrigation. Asymptotic gas production (A), rate constant (c), lag time (L), G_{24} , average

fermentation rate, as well as gas production measured in the presence of polyethylene glycol (PEG₆ and PEG₂₄), did not differ significantly ($p > 0.05$) between sampling before and after irrigation. There was a significant ($p < 0.05$; Table A1) irrigation $\times$ species interaction on tannin activity indices (PEG₆ or PEG₂₄). In *Ac. fimbriata*, tannin activity in terms of PEG₆ was lower before (13.4%) than after irrigation (PEG₆ = 20.0%). In contrast, in *M. arborea* (lowest tannin activity), PEG₆ values were greater before than after irrigation (2.4 vs. not detected), and in *An. barba-jovis*, there was a minor difference (PEG₆ = 5.5 and 4.9% before and after irrigation, respectively). The extent of degradation was numerically greater before than after irrigation, although this marginal difference should be interpreted as a statistical tendency ($p = 0.050$). No significant differences ($p > 0.05$) were observed between before and after irrigation for any of the in vitro digestibility parameters, including D48_{DM}, IVDMD, D144_{DM}, and PTDM.

Table 5. Effect of irrigation (after vs. before (dry) irrigation) on chemical composition, in vitro gas production kinetics and in vitro digestibility.

	Irrigated	Dry	SEM	<i>p</i> -Value
Chemical composition (g/kg DM)				
Ash	71	74	6.8	0.775
Crude protein	146	127	9.2	0.154
Neutral detergent fibre	508	531	27.4	0.545
Acid detergent fibre	364	388	20.6	0.415
Lignin	116	129	6.6	0.201
Gas production kinetics				
A (mL/g DM)	120	116	9.2	0.753
c (per h)	0.052	0.070	0.0074	0.100
L (h)	0.47	0.37	0.149	0.650
Average rate (mL/h)	4.27	4.96	0.420	0.261
G ₂₄ (mL/g DM)	83.8	86.8	7.48	0.780
PEG ₆ (%)	7.11	7.62	1.32	0.788
PEG ₂₄ (%)	6.13	7.55	1.36	0.467
Extent of degradation in the rumen (g/kg DM)	482	521	13.1	0.050
In vitro digestibility (g/kg DM)				
D48 _{DM}	480	444	39.2	0.535
IVDMD	620	593	24.1	0.443
D144 _{DM}	516	519	44.4	0.960
PTDM	814	818	13.1	0.847

Values are means. SEM: standard error of the mean. A: asymptotic gas production; c: fractional rate of gas production; L: lag time; G₂₄: gas production at 24 h of incubation; PEG₆ and PEG₂₄: gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation; D48_{DM}: apparent in vitro digestibility; IVDMD: true in vitro digestibility after NDF treatment; D144_{DM}: dry matter disappearance after 144 h; PTDM: potentially true DM digestibility.

4. Discussion

This study evaluated the chemical composition, in vitro fermentation kinetics, and digestibility of the Mediterranean leguminous shrub species *Ac. fimbriata*, *M. arborea*, and *An. barba-jovis*. The experimental design allowed assessment of the independent effects of species, botanical fraction, season, and irrigation. The findings suggest that forage quality was primarily driven by species, plant part, and seasonal variations, with irrigation showing only a marginal effect. To evaluate the biological activity of tannins, in vitro fermentation assays were run using PEG in the incubation medium. This approach allowed for the determination of the extent to which rumen fermentation of each species could be enhanced through PEG supplementation [4,42].

4.1. Effects of Cultivated Species

The shrub species evaluated in this study are well-adapted to the arid and semi-arid conditions characteristic of Mediterranean environments [43]. While *M. arborea* is a primary woody fodder species cultivated extensively throughout the Mediterranean basin [44], research on the other two species remains limited, despite the availability of data on other shrubby species within the genera *Acacia* and *Anthyllis*.

Modest and rather limited interspecific variations were found in chemical composition. Only *Ac. fimbriata* showed higher ash concentrations than *An. barba-jovis*. This aligns with previous reports of high mineral content in *Acacia* species [24,25]. Such findings suggest that *Ac. fimbriata* could be a potentially valuable source of minerals for ruminants in silvopastoral systems [25]. Conversely, Chouhaira et al. [26] reported that leaves of *An. barba-jovis* had a very low mineral concentration compared to leaves of other leguminous fodder species.

Although the contents of protein and fibre fractions did not differ significantly among species, some differences were observed in fermentation kinetics and digestibility, indicating that nutritive value was influenced more by secondary metabolites and substrate degradability than by proximate composition alone. *M. arborea* consistently showed higher in vitro digestibility and more advantageous fermentation kinetics, indicating potential superior nutritional quality. These results agree with previous studies reporting the high nutritive value and digestive utilisation of *M. arborea* and other Mediterranean leguminous shrubs [18,22,23,45]. Such interspecific variation in nutritive quality may be linked to differential nitrogen availability driven by aridity gradients in Mediterranean environments [46].

Comparing these findings with the existing literature reveals both consistencies and noteworthy divergences that help contextualise the observed interspecific differences. For *M. arborea*, Al-Masri [18] reported CP concentrations of 120–185 g/kg DM and IVDMD values above 600 g/kg DM across several growth stages, which are broadly consistent with the CP values (approximately 150 g/kg DM in leaves, across all treatments) and the superior digestibility of *M. arborea* found in the present study. Similarly, Lambert et al. [47] and Laamouri et al. [23] placed tree medick among the higher-quality fodder shrubs in their respective comparisons, which corroborates the ranking observed herein. For *Ac. fimbriata*, although species-specific published data are limited, values for related *Acacia* species (including *Ac. salicina* or *Ac. karroo*) consistently document reduced fermentability and digestibility when biologically active condensed tannins are present, together with marked positive PEG responses, which mirrors the pattern found here for *Ac. fimbriata* [48,49]. For *An. barba-jovis*, the limited literature available reports moderate tannin concentrations and digestibility values for related species such as *An. vulneraria* [26,27,50], which aligns with the intermediate position of *An. barba-jovis* in the present study. Where present-study values differ quantitatively from those in the literature, the most plausible explanatory factors include the Tunisian semi-arid climate (with its more pronounced summer drought and higher temperatures compared with the Atlantic or oceanic climates of some reference studies, favouring earlier and stronger lignification), the advanced plant age at sampling (fourth year of growth, with well-established woody stems), and the specific cutting management applied (sporadic irrigation only, regular cutting, or grazing regimes in some referenced experiments), all of which tend to increase fibre content and reduce fermentability relative to younger, more intensively managed shrubs.

The response to PEG addition clearly differentiated the species. Gas production in response to the addition of PEG (at 6 or 24 h of incubation) was markedly higher for *Ac. fimbriata*, whereas with *M. arborea* only a minimal response was observed. Since PEG selectively binds condensed tannins and lessens their inhibitory effects on ruminal fermentation, these results indicate the presence of biologically active tannins in *Ac. fimbriata*, whereas tan-

nin concentrations or biological activity in *M. arborea* would be most likely negligible [7,51]. Aerial parts of tree medick (*M. arborea*) are rich in saponins but contain only marginal levels of tannin compounds [28]. Chouhaira et al. [26] reported moderate total condensed tannin contents in leaves of *An. barba-jovis*, whereas other phytochemical screenings [27] revealed minor tannin concentrations in related *Anthyllis* species (*An. vulneraria*). In contrast, foliage from Acacia trees is known to contain high levels of polyphenol compounds, in particular condensed tannins [48,49,52,53].

Acacia foliage is predominantly characterised by condensed tannins (proanthocyanidins) rather than hydrolysable tannins, which is consistent with the marked PEG response observed for *Ac. fimbriata*, since condensed tannins are known to bind readily to dietary and microbial proteins under ruminal pH conditions [7]. This distinction is relevant because condensed and hydrolysable tannins differ in their biological consequences: hydrolysable tannins can be degraded to potentially toxic phenolic and tannic acid derivatives, whereas condensed tannins are generally considered safer for ruminants at moderate concentrations and are associated with a recognised dual effect on production. On one hand, the protein-binding action of condensed tannins that depressed fermentation in the present PEG assay can, at adequate dietary concentrations, also protect dietary protein from excessive ruminal degradation, increasing the flow of bypass protein to the small intestine [12]. On the other hand, the same tannins have been associated with anthelmintic activity against gastrointestinal nematodes such as *Haemonchus contortus* [12], a feature of particular relevance for small-ruminant systems in Mediterranean drylands where parasitism is a major production constraint. These dual properties suggest that, rather than being excluded from feeding programmes on the basis of lower fermentability alone, *Ac. fimbriata* could be strategically incorporated at moderate inclusion levels or alternated with low-tannin forages such as *M. arborea* to balance digestibility against the agronomic and parasite-control benefits associated with its tannin content.

In vitro dry matter and fibre digestibility further supported these differences. *Ac. fimbriata* was the least digestible fodder despite comparable fibre contents. The lower digestibility of *Ac. fimbriata* may therefore be attributed to the combined effects of tannin activity and higher ash content, which can reduce the proportion of fermentable organic matter available to rumen microbes [49,54,55]. Similar relationships between secondary compounds and digestibility have been reported previously [49,56,57]. In contrast, *M. arborea* has been shown to provide fodder of relatively high nutritive value to small ruminants owing to its high digestibility [23,47]. In line with data reported for other *Anthyllis* species [50], the digestibility of fodder from *An. barba-jovis* seems to be comparable to that from tree medick.

4.2. Effects of Plant Part

Botanical fraction (the plant part, leaves or twigs) was the most influential factor affecting forage quality. Leaves consistently showed higher crude protein and lower fibre concentrations (NDF, ADF, and ADL) than stems, resulting in greater in vitro gas production, extent of degradation, fermentation rates, and digestibility. This pattern reflects a lower degree of lignification and greater accessibility of cell wall carbohydrates in leaf tissues, which facilitates microbial attachment and enzymatic degradation [19,31]. Leaves represent the primary site of metabolic activity and protein synthesis, leading to significantly higher CP concentrations and lower fibre fractions compared to stems. In Mediterranean and tropical leguminous shrubs, such as *M. arborea* or *Leucaena leucocephala*, leaves consistently contain 1.5 to 3 times more nitrogen than the twigs or stems of the same species [58–60]. In contrast, stems function mainly as structural support and are characterised by advanced lignification.

Differences in chemical composition are associated with divergent fermentation kinetics and in vitro digestibility. When incubated in buffered rumen fluid, the leaf fraction is fermented to a greater extent, resulting in increased cumulative gas production compared to stems, which contain more fibre and less readily fermentable carbohydrates than leaves [31,60]. Similar differences between leaves and stems have been reported for other leguminous plants and cultivars [61–64]. The more developed and lignified stem cell walls create a physical barrier that restricts microbial access during digestion [5], thereby hampering the attachment and cellulolytic activity of rumen bacteria and fungi and reducing fermentability [65,66]. Thus, stems are characterised by limited nutritive value and lower digestibility than leaves due to their higher content of structural carbohydrates and lignin.

The leaf-to-stem ratio of leguminous browse plants is the most influential factor determining their feeding value for small ruminants [29,30], but this parameter was not quantified in the present study. Although analysing a composite sample can provide an estimate of whole-shoot nutritive value, this approach requires a highly representative and stable leaf-to-stem ratio. In practice, a single composite ratio is rarely representative across diverse conditions; without a highly standardised, repeatable sampling protocol, it can introduce substantial experimental bias. Furthermore, even an accurately determined leaf-to-stem ratio yields results valid only for that specific instance, as this parameter varies dynamically with season, irrigation status, shoot maturity, canopy position, and phenological stage. Consequently, a single whole-shoot sample cannot reliably represent the canopy biomass available for browsing under any circumstances. To circumvent this limitation, leaves and twigs were separated and analysed as independent fractions rather than being weighed as fixed proportions of the intact shoot. Our results clearly demonstrate that these two fractions differ markedly in chemical composition and digestibility. By establishing these baseline values for the individual components, the overall nutritive value of the browsing material can be mathematically estimated for any given scenario if the specific leaf-to-stem ratio is known (i.e., as a weighted whole-shoot nutritive value). The observations detailed in this discussion regarding the influence of the leaf-to-stem ratio on overall feeding value rely on context from published literature rather than on primary data generated herein.

From a harvesting and utilisation standpoint, the pronounced differences between leaf and stem fractions in the present study suggest concrete management implications for Mediterranean silvopastoral systems. The leaf fraction of all three species delivered substantially greater fermentable energy and digestible protein per unit of dry matter than the stem fraction, which means that maximising the leaf proportion in the diet has a disproportionately large positive effect on ruminant nutrient supply. In practice, this can be achieved through selective browsing management (limiting access time to prevent excessive woody stem consumption), cut-and-carry operations that target the leafy canopy rather than whole-shoot harvesting, or leaf stripping prior to feeding when material is harvested mechanically. For species with high tannin concentrations in leaves, such as *Ac. fimbriata*, the high leaf PEG response observed here indicates that maximising leaf intake from this species alone may not improve nutrient supply proportionally, because tannin activity partially offsets the inherent compositional advantage of the leaf over the stem; however, when *Ac. fimbriata* is offered alongside low-tannin species such as *M. arborea*, diet-level tannin dilution should reduce the anti-nutritional effect while preserving its potential anthelmintic contribution. These considerations illustrate how species-specific knowledge of leaf and stem quality, as generated in the present study, can directly inform practical harvesting and allocation decisions in silvopastoral systems. The higher PEG₆ and PEG₂₄ values observed in leaves suggest that tannins, when present, are mainly concentrated in

foliage rather than in stem fractions, as previously reported for Mediterranean shrubs [58]. Tannins are often concentrated in the leaves of certain species to deter herbivory [58].

4.3. Effects of Season

Seasonal variations influenced fibre composition, fermentation kinetics, and digestibility. Fodder harvested in spring consistently showed better fermentation kinetics and digestibility, whereas autumn-collected material was characterised by higher NDF, ADF, and ADL contents. These seasonal trends are consistent with previous studies reporting increased fibre accretion and lignification with advanced plant maturity and after exposure to summer drought stress [31,67,68].

The most pronounced seasonal change reported in the literature for leguminous browse plants is the decline in leaf-to-stem ratio (this parameter was not measured in the present study). This ratio is higher in spring when leaf growth is more accelerated and decreases steadily as the tissues become more mature. Beyond quantitative changes in fodder composition (proportions of leaf and stem mass), seasonal differences in fermentability are closely associated with modifications in cell wall structure. The most digestible soluble fractions reach higher proportions during periods of active plant growth [69]. Autumn vegetation has experienced prolonged environmental stress and evolved to more mature tissues, leading to increased cell wall complexity and reduced fermentability. Even small seasonal changes in cell wall lignification can substantially affect fibre digestibility, as highlighted by Colas et al. [70].

In semi-arid ecosystems, the transition from spring to autumn involves a substantial physiological shift in leguminous shrubs, primarily driven by water availability and phenological ageing. During the spring, the combination of residual winter soil moisture and rising ambient temperatures triggers the “flush” of growth of new leaves and shoots [71,72]. With the onset of favourable spring conditions, the browse species recover from metabolic quiescence and initiate a vegetative growth phase with high metabolic activity. This causes the emergence of new leaves and the elongation of new terminal shoots [71,73]. These young tissues contain high concentrations of protein and thinner and more loosely packed cell walls prior to the onset of lignification [22,31]. In Mediterranean leguminous shrubs like *M. arborea* and *Cytisus* species, spring growth has significantly lower levels of structural cell wall components, resulting in enhanced microbial fermentation and digestibility [31,74]. Ventura et al. [48] observed that seasonal effects were highly variable depending on the forage shrub species, but no interaction between season and species was observed in our study for any of the variables analysed, most likely because only leguminous species were evaluated.

In contrast, as the season progresses into autumn, the cumulative effect of high summer temperatures and prolonged water scarcity induces progressive tissue lignification. The plant redirects resources from primary growth to structural integrity and survival, leading to a marked increase in cell wall carbohydrates and lignin in both leaves and stems [22,74–76]. In leguminous shrubs, water stress in autumn may also trigger the accumulation of secondary metabolites such as condensed tannins, but this was not observed in our study. The digestibility of these plants is inversely proportional to their fibre content, which peaks in the late dry season (autumn). In leguminous browse plants, at the end of the summer, the stems become highly lignified, whereas leaves may retain a moderate protein level to maintain basic metabolic functions. However, the interaction plant part by season was not significant for any of the chemical or nutritional quality parameters assessed.

4.4. Effects of Irrigation

In arid and semi-arid regions, water availability (either through natural rainfall or by irrigation) may affect the nutritive value of forage and browse plants. Severe water stress can reduce plant height, leaf area, and leaf-to-stem ratio [77]. While water availability in leguminous plants increases biomass yield, it may also dilute nitrogen concentration in the plant tissues [78,79]. Effects on cell walls and fibre content are more uncertain because irrigation may hasten lignification due to rapid plant growth, but water deficit may also lead to drought-induced maturation, increasing the proportion of senescent fibrous tissues [78,80,81].

In our study, irrigation had no significant effects on chemical composition, *in vitro* gas production, or digestibility. These results suggest that, in shrub species well adapted to Mediterranean environments, water availability primarily affects quantitative traits such as biomass yield rather than intrinsic forage quality [79,80]. Similar findings have been reported by others [17,82,83], who observed that irrigation improves productivity without substantially modifying nutritional characteristics. From a physiological standpoint, several mechanisms may explain why short-term changes in water availability had limited impact on intrinsic forage quality in these cultivated shrubs. First, the deep-rooting systems characteristic of Mediterranean Fabaceae species [10,11] allow access to subsoil moisture reserves that buffer xylem water potential against short-term surface irrigation events so that a single watering episode is unlikely to substantially alter whole-plant water status in mature, drought-adapted individuals. Second, nutrient allocation in woody perennials is governed largely by source-sink relationships established over the growing season rather than by immediate water pulses; nitrogen and carbon investment in newly formed tissue is therefore more closely tied to phenological stage (and thus to season, which did show significant effects) than to short-term irrigation status [84]. Third, the biosynthesis of condensed tannins and other phenolic compounds is regulated primarily by the carbon-to-nitrogen balance and by longer-term drought-acclimation signalling pathways, including acid-mediated responses, rather than by transient changes in soil moisture [85], which is consistent with the negligible irrigation effect observed here on PEG₆ and PEG₂₄. Taken together, these mechanisms suggest that the chemical and fermentative quality of leaves and stems in these species is buffered against short-term water pulses while remaining sensitive to the larger and more sustained physiological shifts associated with seasonal phenology. The ability of these shrubs to maintain relatively stable nutritive value under drought conditions reinforces their suitability for sustainable forage production in water-limited environments.

4.5. Limitations and Future Research Directions

Several limitations should be acknowledged when interpreting the results presented herein. First, the assessment relied exclusively on *in vitro* techniques, which, while consistent with the 3Rs principles and well-suited to screening multiple species and treatment combinations, cannot fully capture animal-level responses such as voluntary intake, post-ruminal digestion, or the *in vivo* physiological consequences of tannin ingestion. Therefore, further *in vivo* feeding trials are required before firm recommendations on inclusion levels can be made. Second, sampling was restricted to a single annual cycle (2008), so that the seasonal and irrigation effects reported here reflect the particular meteorological conditions of that year (Table 1) and may not fully represent year-to-year climatic variability typical of Mediterranean drylands; multi-year monitoring would strengthen confidence in the generality of the seasonal patterns observed. Third, tannin activity was inferred indirectly from the PEG bioassay rather than from direct quantification of tannin content (albeit most available methods are not fully quantitative). Whereas the PEG approach has the advantage

of capturing the net biological activity of the phenolic fraction as it affects fermentation, it does not establish the absolute concentration or precise chemical class of tannins present, and complementary quantitative analyses would help characterise the active compounds more fully. Building on these limitations, future research should prioritise in vivo feeding trials with small ruminants to validate the fermentation and digestibility rankings reported here; multi-year seasonal monitoring across additional rainfall years to test the robustness of the season and irrigation effects; direct quantification of condensed tannin content and composition in parallel with PEG bioassays; and evaluation of mixed-species or mixed-part feeding strategies (for example, combining *Ac. fimbriata* with a low-tannin species) to determine whether the anthelmintic and protein-protective benefits of condensed tannins can be captured without a net loss of overall diet digestibility.

5. Conclusions

Among the three leguminous shrub species evaluated, *M. arborea* demonstrated the highest nutritive value, followed by *An. barba-jovis*. In contrast, *Acacia fimbriata* showed comparatively lower digestibility and nutritive value, though it can still be considered a suitable supplementary feed for small ruminant consumption within silvopastoral systems, particularly under conditions where alternative forages are limited. Fodder quality was strongly influenced by both phenological stage and botanical plant part. Fodder harvested in spring is consistently of superior nutritional value compared with that collected in autumn. The most influential factor was the plant part, with leaves remaining more digestible than stems across all species. Irrigation has only a marginal impact on the chemical composition and digestibility of the fodder, regardless of the species and season, indicating that these shrubs maintain relatively stable feeding value under variable water availability. From a practical standpoint, *M. arborea* is recommended as the priority species for direct browsing or cut-and-carry feeding wherever establishment is feasible, given its consistently high digestibility and minimal tannin-related fermentation constraints. *An. barba-jovis* represents a comparable second-choice fodder resource, whereas *Ac. fimbriata*, owing to its active condensed tannins, is best positioned as a supplementary rather than primary forage, with potential added value as a natural anthelmintic component when included at moderate, controlled levels in mixed diets. In terms of harvest management, spring harvesting and preferential utilisation of the leaf fraction (through selective browsing or leaf stripping prior to feeding) should be prioritised whenever the production objective is to maximise fermentable energy and digestible nutrient supply, while autumn or stem-rich material remains usable as a lower-quality maintenance feed when higher-quality forage is scarce. Given the negligible effect of irrigation on intrinsic nutritive value, supplementary irrigation of these established shrubs does not appear necessary purely to preserve forage quality, although it may still be justified where the objective is to increase total biomass yield rather than nutritive value per unit of forage. Where the tannin content of *Ac. fimbriata* limits its fermentability, PEG supplementation, or alternative tannin-mitigation strategies such as tannin-tolerant rumen inocula or blending with low-tannin forages, could be evaluated as practical means of improving its feeding value without forfeiting its anthelmintic potential. These findings underscore that the strategic selection of species, combined with a focus on seasonal utilisation and leaf-to-stem ratios, is essential for maximising the potential of these shrubs as sustainable feed resources for small ruminant production.

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Abbreviations

The following abbreviations are used in this manuscript:

A	Asymptotic gas production
ADF	acid detergent fibre
ADL	acid detergent lignin
CP	crude protein
<i>c</i>	fractional rate of gas production (h^{-1})
CO_2	carbon dioxide
$\text{D}_{48\text{DM}}$	apparent in vitro digestibility (DM disappearance from the bag after 48 h of incubation in buffered rumen fluid)
$\text{D}_{144\text{DM}}$	DM disappearance from the bag after 144 h of incubation in buffered rumen fluid
DM	dry matter
G_{24}	gas production at 24 h of incubation
GP	gas production
IVDMD	true in vitro DM digestibility (after 48 h of incubation in buffered rumen fluid followed by NDF extraction)
<i>L</i>	lag time (h)
NDF	neutral detergent fibre
PEG	polyethylene glycol
PEG_6	gas production increase upon the addition of polyethylene glycol measured at 6 h of incubation
PEG_{24}	gas production increase upon the addition of polyethylene glycol measured at 24 h of incubation
PTDMD	potentially true DM digestibility (after 144 h of incubation in buffered rumen fluid followed by NDF extraction)
SEM	standard error of the mean

Appendix A

Table A1. Level of significance (*p*-values) of all the two-way interactions between the four sources of variation in the full (preliminary) ANOVA model.

	Interaction					
	Species × Season	Species × Plant Part	Species × Irrigation	Season × Plant Part	Season × Irrigation	Plant Part × Irrigation
Chemical composition (g/kg DM)						
Ash	0.67	0.38	0.06	0.14	0.87	0.62
Crude protein	0.91	0.75	0.85	0.98	0.54	0.33
Neutral detergent fibre	0.10	0.58	0.18	0.55	0.64	0.61
Acid detergent fibre	0.11	0.40	0.32	0.53	0.36	0.30
Lignin	0.54	0.45	0.12	0.83	0.22	0.34
Gas production kinetics						
A (mL/g DM)	0.92	0.50	0.52	0.57	0.86	0.94
c (per h)	0.45	0.56	0.49	0.78	0.30	0.18
L (h)	0.81	0.46	0.78	0.87	0.74	0.82
Average rate (mL/h)	0.99	0.65	0.20	0.77	0.62	0.39
G ₂₄ (mL/g DM)	0.96	0.53	0.35	0.75	0.90	0.73
PEG ₆ (%)	0.05	0.02	0.01	0.92	0.80	0.16
PEG ₂₄ (%)	0.50	0.06	0.02	0.47	0.48	0.64
Extent of degradation in the rumen (g/kg DM)	0.56	0.69	0.21	0.82	0.29	0.14
In vitro digestibility (g/kg DM)						
D48 _{DM}	0.57	0.98	0.74	0.83	0.84	0.73
IVDMD	0.30	0.91	0.84	0.63	0.71	0.61
D144 _{DM}	0.76	0.79	0.63	0.44	0.88	0.96
PTDMD	0.80	0.73	0.85	0.77	0.86	0.88

A: asymptotic gas production (mL/g DM); c: fractional rate of gas production (h⁻¹); L: lag time (h); G₂₄: gas production at 24 h of incubation (mL/g DM); PEG₆ and PEG₂₄: gas production increase upon the addition of polyethylene glycol measured at 6 and 24 h of incubation; D48_{DM}: apparent in vitro digestibility; IVDMD: true in vitro digestibility after NDF treatment; D144_{DM}: dry matter disappearance after 144 h; PTDMD: potentially true DM digestibility.

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