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Original Article

Seasonal Quality Assessment of Leaves and Stems of Fodder Ligneous Species

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Abstract

The objective of this research was to investigate the effect of seasonality on the chemical composition and concentration of phenolic compounds in some ligneous species. The research was conducted at the Aristotle University's farm, Thessaloniki, Greece. From five ligneous species (*Robinia pseudoacacia* var. *monophylla*, *Amorpha fruticosa*, *Colutea arborescens*, *Morus alba* and *Arbutus unedo*) samples (leaves and twigs) were collected during two different seasons (spring and autumn). All samples were analyzed for crude protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Lignin (ADL) and *in vitro* dry matter (DM) digestibility (IVDMD), as well as for total phenols (TPH), total tannins (TT) and condensed tannins (CT). The CP content was generally lower and higher in leaves and stems of *A. unedo* and *R. pseudoacacia*, respectively compared to the other tested species. *A. unedo* had the highest values in NDF and ADL in leaves and the highest ADL content in stems. *M. alba* and *C. arborescens* in leaves and *C. arborescens* in stems had the lowest values of ADL content. *M. alba* had the significantly highest value in IVDMD and *A. unedo* the lowest one in both leaves and stems. Season had no significant effect on TPH, TT, and CT of leaves, while in stems they were significantly higher in autumn. *A. unedo* had the highest concentration of TPH, TT and *R. pseudoacacia* of CT in the leaves. TPH, TT, and CT concentration in stems was significantly higher in *A. unedo*. According to the findings, the impact of maturation on the type and the concentration of phenols and tannins is species-dependent.

Keywords: browse species; Mediterranean region; nutritive value; phenological stage; tannins

Abbreviations: ADL - acid detergent lignin expressed exclusive of residual ash; NDF - neutral detergent fiber expressed inclusive of residual ash; CP - crude protein; DM - dry matter; IVDMD - in vitro DM digestibility; MPTS - Multipurpose trees and shrubs; CT - condensed tannin; TPH - total phenols; TT - total tannins; PVPP - polyvinyl-polypyrrolidone; NTP - non-tannin phenols; TAE - tannic acid equivalent; QE - quebracho equivalent.

Introduction

Multipurpose trees and shrubs (MPTS) could be a valuable alternative green forage source for grazing animals throughout the year (evergreen species) or during critical periods (deciduous ones) (Kokten *et al.*, 2012; Barakat *et al.*, 2013). It is well documented that the leaves and twigs of woody species constitute the main resources in diet of goats grazing in the Mediterranean area (Papachristou and Nastis, 1996; Manousidis *et al.*, 2016). Moreover, the use of branches and leaves for making a foliar fodder is still practiced in many European countries, especially in the Balkan peninsula to provide roughages for livestock during the winter. The usage of browse species' foliage could be efficiently contributed to the reduction of ruminal methanogenesis in livestock species fed low-quality forage diets and to improve their performance (Delgado *et al.*, 2012).

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However, their potential as feed resources by herbivores is often restricted by defending or deterring mechanisms related to phenolic compounds (particularly high condensed tannin (CT) content) (Provenza, 1995; Rubanza *et al*., 2003; Bakshi and Walhwa, 2004; Sallam *et al*., 2010) which decrease feed intake, nutrient digestibility and nitrogen retention (Kumar and Vaithiyanathan 1990; Silanikove *et al*., 1996; Bansi *et al*., 2014). Tannins have differential effects (Rana *et al*., 2006) on animals ranging from beneficial to toxicity and even death (Makkar, 2003a) mainly depending on their concentration and nature. Thus, *Lotus corniculatus* (CT 18-29 g kg^{-1} DM) in ewes' diet it was found to increase animal productivity and reproduction efficiency (Min *et al*., 2001; Ramirez-Restreppo *et al*., 2005), while *Lotus pedunculatus* (CT content > 50 g kg^{-1} DM) had a negative effect on voluntary feed intake of grazing sheep (Barry and McNabb, 1999). Moreover, consumption of *Terminalia oblongata* leaves with high hydrolysable tannins concentration caused poisoning of sheep (McSweeney *et al*., 2001).

Chemical composition of woody species is significantly influenced by season (Evans, 1989; Singh and Todaria, 2012) and the nutritive value is generally decreased due to physiological changes during the growing season (Al-Masri, 2013). This decrease over the growing season is mainly attributed to the dilution effect. It is known that leaves contain more than 40% of the total nitrogen in a browse species (Kramer and Kozlowski, 1979) and their nutritive value is higher compared to the stems (Cordesse *et al*., 1991). This difference in their quality is became higher as the plant matures. Similarly, the concentration of tannins is influenced by maturity stage, leaf age and and/or collection season and proportion of sample's foliage (Schultz *et al*., 1982; Rogler and Sell, 1984; Salem, 2005). For instance, Paul *et al.* (1991) found higher total extractable phenols in younger leaves of *Quercus semecarpifolia* in comparison to old ones.

Browse species have been studied from many researchers especially in the Mediterranean region as they are essential components of the native vegetation (Gilboa *et al*., 2000; Corleto *et al*., 2009). These species either indigenous as *Quercus coccifera, Fraxinus ornus, Carpinus orientalis, Arbutus unedo, Colutea arborescens* or introduced as *Robinia pseudoacacia, Morus alba, Amorpha fruticosa* (Papachristou and Papanastasis, 1994; Papachristou *et al*., 1999; Parissi, 2001; Roukos 2016) are characterized as valuable feed resource for livestock. However, there is limited research conducted on the effect of growth stage, particularly in relation to the anti-nutritional components in leaves and stems of browses used as a feed for ruminants. Therefore, the objective of this research was to investigate the effect of seasonality (vegetative and maturity stage) on the chemical composition and concentration of phenolic compounds in leaves and stems of some ligneous species.

Materials and Methods

Study area and plant materials

Five ligneous species [the deciduous leguminous species *Robinia pseudoacacia* var. monophylla (L.) (R.ps.), *Amorpha fruticosa* L. (A.fr.), *Colutea arborescens* L. (C.ar.), the deciduous *Morus alba* L. (M.al.), and the evergreen *Arbutus unedo* L. (A.un.)] were investigated in this study. All species were kept in a shrubby form as they cut in 60 cm height every January. All browse samples were been harvested from plantations in Aristotle University's farm (40° 34' E, 23° 43' N, at sea level) in northern Greece. The climate of the area is defined as Mediterranean semiarid with cold winters. The mean annual temperature and precipitation are 15.5 °C and 443 mm, respectively.

Sample collection and chemical analyses

Samples were hand-plucked in 2009 (i.e., leaves and twigs with diameter <3mm) from three individual plants per species at two different periods and stages of maturity as follows: (i) during the season of rapid growth (immature: May, IM) when the leaves were young, and (ii) in September (mature: September, M) when growth had ceased and the woody parts had been hardened. Upon collection, each plant sample was divided into leaves and stems and oven-dried at 50 °C for 48 h. All samples were ground through a 1 mm sieve and were analyzed for N using a Kjeldahl procedure (method 2001.11 of the AOAC, 2002) and crude protein (CP) was calculated as N content × 6.25. Plant fiber analysis was performed according to the method of Van Soest *et al.* (1991), for Neutral Detergent Fiber (NDF) and Acid Detergent Lignin (ADL).

The analyses for total phenols (TPH), total tannins (TT) and condensed tannins (CT) were completed in three replicates according to Makkar (2003b). For the plant extraction, approximately 200 mg of ground plant sample was been weighed and inserted in a 25 ml glass tube. 10 ml of aqueous acetone (70%) added to the tube and suspended in an ultrasonic water bath for 20 min (2×10 min, with 5 min break) at room temperature. Subsequently, the tube was been centrifuged for 10 min at 3000×g at 4 °C and the supernatant was collected. This procedure repeated three times per sample. The TPH and TT determined by a modified Folin-Ciocalteu method using polyvinyl-poly-pyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (NTP). Between 0.02 and 0.1 ml of the extract (depending on the concentration of TP and TT in the plant sample) was put in a test tube and filled up to the volume of 0.5 ml with distilled water. Folin-Ciocalteu reagent (0.25 ml; 1N; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 1.25 ml sodium carbonate solution (40 g $Na_2CO_3{\cdot}10H_2O$ in 200 ml distilled water) were added. The tube was vortexed and absorbance for TP was been recorded at 725 nm after 40 min using a UV-vis spectrophotometer. For measuring TT, 100 mg PVPP (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in a test tube was added by 1.0 ml distilled water and 1.0 ml of the extract. The mixture was vortexed and kept at 4 °C for 15 min, centrifuged (3000×g, 4 °C, 10 min), and the supernatant, containing only the NTP, was decanted. Measurement of the phenolic content of the supernatant was as described above. Both TP and TT were calibrated against tannic acid solution as a standard (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and values as tannic acid equivalent (mg/g TAE) (Makkar *et al*., 1993). Condensed tannins (CT) were determined according to the method of Porter *et al.* (1986), using purified quebracho as the reference standard and therefore expressed as quebracho equivalent (QE).

In vitro dry matter (DM) digestibility (IVDMD) of the species was determined using a modification of Moore's (1970) and Harris' (1970), Tilley and Terry (1963) two stage digestion method. The rumen liquor was obtained from three ruminally fistulated goats grazing in rangelands, with free access to drinking water and mineral licks. Rumen liquid was collected before the morning meal in thermo-flasks.

Statistical analyses

Two-way ANOVA of data was performed using SPSS® statistical software v. 18.0 (SPSS Inc., Chicago, IL, USA) in order to determine the differences among the tested species in the two stage of maturity in leaves and stems. The Least Significant Difference (LSD) at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

Results

The season significantly affected NDF, ADL and CP contents as well as the IVDMD of leaves and stems in the tested species (Tables 1 and 2). In autumn, the NDF and ADL content for both leaves and stems increased, while CP and IVDMD decreased significantly. The increase of NDF and ADL in autumn was more important in stems than leaves. Particularly, the ADL content of stems in autumn was on average 85% more than in spring, while in leaves was only 15%. As a consequence, the IVDMD decreased on average in leaves only by 5% in autumn but in stems by 40%.

Significant differences in NDF, ADL, CP and IVDMD (Tables 1 and 2) were recorded for both leaves and stems among the tested species across season. In particular, the CP content was generally lower and higher in leaves and stems of *A. unedo* and *R. pseudoacacia* respectively compared to the other tested species (Tables 1 and 2). The CP content ranged from 101 to 273 g kg^{-1}DM (Table 1) in leaves, while from 80 to 147 g kg^{-1}DM in stems (Table 2).

Arbutus unedo had the significantly highest values of NDF and ADL in leaves and the highest ADL content in stems. NDF content in stems of *A. unedo* and *C. arborescens* were higher than *R. pseudoacacia* and *A. fruticosa*. On the other hand, the lowest values of NDF were recorded in *M. alba* and *A. fruticosa* in leaves and stems respectively. Regarding the ADL, *M. alba* and *C. arborescens* in leaves and *C. arborescens* in stems had the lowest values. Finally, *M. alba* had the significantly higher value in IVDMD and *A. unedo* the lower one in both leaves and stems. Significant interactions were observed between the season and the tested species for all nutritive parameters, with the exception of IVDMD in leaves (Tables 1 and 2), indicating that the effect of season was not consistent for all the species. The NDF content in leaves of *A. fruticosa* and of *C. arborescens* did not significantly differ between autumn and spring (Fig. 1).

Similarly, the ADL and CP content in leaves of *M. alba* (Fig. 1), the CP of C. *arborescens* and of *A. unedo* (Fig. 1)

Table 1. The effect of season and species on chemical composition (g kg^{-1}DM) and IVDMD (g kg^{-1}DM) of browsed leaves (mean ± SE)

Season	CP (g kg^{-1}DM)	NDF (g kg^{-1}DM)	ADL (g kg^{-1}DM)	IVDMD (g kg^{-1}DM)
Spring	247±25a*	276±26b	61±12b	590±6a
Autumn	187±16b	307±31a	70±12a	560±6b
Species				
Robinia pseudoacacia	273±30a	266±15c	56±7c	572±14c
Amorpha fruticosa	232±43bc	327±6b	97±2b	454±12d
Morus alba	219±3c	211±13d	32±1d	811±14a
Arbutus unedo	108±12d	436±19a	115±4a	319±10e
Colutea arborescens	254±9ab	219±5d	28±3d	718±32b
Source of variation				
Season (A)	P<0.05	P<0.05	P<0.05	P<0.05
Species (B)	P<0.05	P<0.05	P<0.05	P<0.05
A×B (Interaction)	P<0.05	P<0.05	P<0.05	ns

*Means followed by the same letter in the column for each independent variable did not significantly differ (P>0.05); **ns: not significant.

Table 2. The effect of season and species on chemical composition (g kg^{-1}DM) and IVDMD (g kg^{-1}DM) of browsed stems (mean ± SE)

Season	CP (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADL (g kg^{-1}DM)	IVDMD (g kg^{-1}DM)
Spring	149±18a	581±19b*	78±10b	580±51a
Autumn	78±5b	692±5a	145±13a	351±30b
Species				
Robinia pseudoacacia	147±36a	616±58b	117±33b	478±103b
Amorpha fruticosa	122±22a	612±49b	125±31b	498±32b
Morus alba	137±36a	633±34ab	92±17c	617±94a
Arbutus unedo	80±14b	663±10a	155±11a	277±27c
Colutea arborescens	81±4b	659±16a	69±6d	458±80b
Source of variation				
Season (A)	P<0.05	P<0.05	P<0.05	P<0.05
Species (B)	P<0.05	P<0.05	P<0.05	P<0.05
A×B (Interaction)	P<0.05	P<0.05	P<0.05	P<0.05

*Means followed by the same letter in the column for each independent variable did not significantly differ (P>0.05); **ns: not significant.

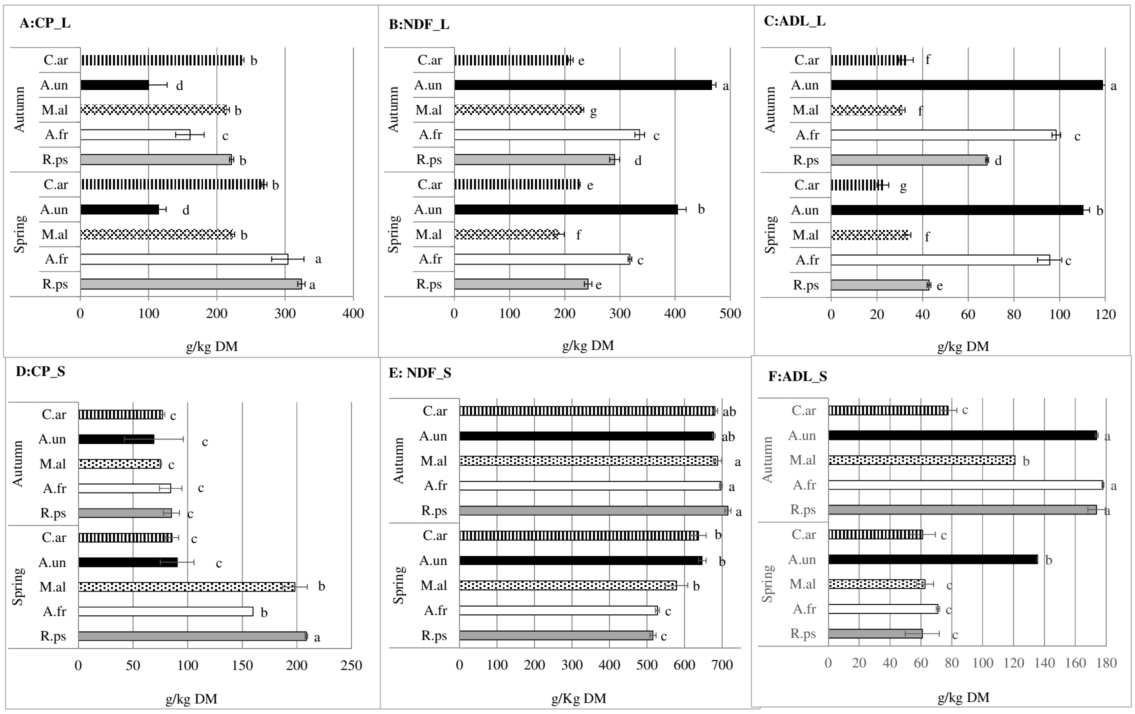

Fig. 1. Interactions between season × browse species for CP (g kg^{-1}DM), NDF (g kg^{-1}DM) and ADL (g kg^{-1}DM) content, for leaves (L) and stems (S). R.ps.: *Robinia pseudoacacia,* A.fr.: *Amorpha fruticosa*, C.ar., *Colutea arborescens*, M.al.: *Morus alba*, A.un.: *Arbutus unedo*. *Means followed by the same letter for the same content did not significantly differ (P>0.05).

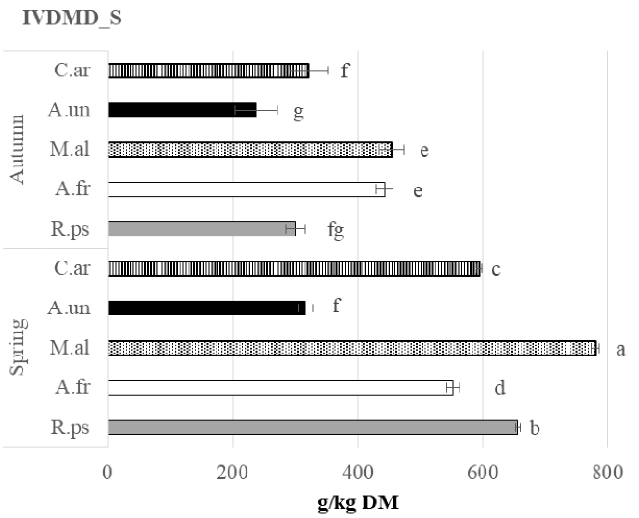

Fig. 2. Interaction between browse species and growth stage of IVDMD (g kg^{-1}DM) in stems (S). R.ps.: *Robinia pseudoacacia*, A.fr.: *Amorpha fruticosa*, C.ar., *Colutea arborescens*, M.al.: *Morus alba*, A.un.: *Arbutus unedo*

and the ADL content of *A. fruticosa* did not significantly differ between autumn and spring. No differences were detected between seasons in the CP and NDF contents in stems of *C. arborescens* and *A. unedo* and in the ADL stem content of *C. arborescens* (Fig. 1). Finally, the IVDMD of *M. alba* in stems was significantly higher in spring and autumn, followed by *R. pseudoacacia* in spring and *A. fruticosa* in autumn (Fig. 2).

Season had no significant effect on TPH, TT, and CT of leaves of the tested species, while it significantly affected the content of the stems (Tables 3 and 4). The TPH, TT, and CT concentrations of stems were significantly higher in autumn compared to spring. Overall, species had significant effect in both TPH, TT, and CT concentration of leaves and stems. Particularly, *A. unedo* had the highest concentration of TPH, TT in the leaves compared to the other species followed by *R. pseudoacacia* and *A. fruticosa*.

On the other hand, *R. pseudoacacia* had the highest concentration of CT in the leaves compared to that of the other species followed by *A. fruticosa* and *Ar. unedo*. Regarding the TPH, TT and CT concentrations in stems, these were significantly higher in *A. unedo* followed by *A. fruticosa*, compared to the others. In all cases, the lowest values were recorded in *C. arborescens* and *M. alba* (Tables 3 and 4). It has also noticed that in all tested species the concentration of TPH, TT, and CT was much lower in stems than in leaves.

There was a significant interaction between season × species for TPH, TT and CT (Table 5) for both leaves and stems. The TPH and TT concentrations in leaves of *R. pseudoacacia* and the TPH of *M. alba* were significantly lower in autumn compared to spring (Table 5), while there were not significant differences between spring and autumn for all the other species.

On the other hand, the CT concentration in leaves of *R. pseudoacacia* and *A. fruticosa* decreased significantly, while this of *A. unedo* increased from spring to autumn. The concentration of TPH, TT and CT in stems of all the tested species generally was immutable or decreased from spring to autumn except of *A. unedo* that was significantly increased (Table 5). Based on their chemical composition, IVDMD and the concentration of condensed tannins the browse species could be ranked as follow: *M. alba* > *R. pseudoacacia* > *C. arborescens* > *A. fruticosa*> *A. unedo*.

Table 3. TPH (mgg^{-1} DM TAE), TT (mgg^{-1} DM TAE), CT (mgg^{-1} DM QE) concentration in leaves as affected by browse species and growth (mean ± SE)

Season	TPH (mgg^{-1} DM TAE)	TT (mgg^{-1} DM TAE)	CT (mgg^{-1} DM QE)
Spring	19±2a*	15±1a	38±6a
Autumn	18±2a	14±1a	34±5a
Species			
Robinia pseudoacacia	23±1b	20±1b	92±5a
Amorpha fruticosa	17±1c	15±1c	54±5b
Morus alba	9±1d	6±0.4d	1±0.03d
Arbutus unedo	34±1a	28±1a	32±4c
Colutea arborescens	7±0.3d	4±0.2d	1±0.07d
Source of variation			
Season (A)	ns	ns	ns
Species (B)	P<0.05	P<0.05	P<0.05
AXB (Interaction)	P<0.05	P<0.05	P<0.05

*Means followed by the same letter in the column for each independent variable did not significantly differ (P>0.05); **ns: not significant.

Table 4. TPH (mgg^{-1} DM TAE), TT (mgg^{-1} DM TAE), CT (mgg^{-1} DM QE) concentration in stems as affected by browse species and growth stage (mean ± SE)

Season	TPH (mgg^{-1} DM TAE)	TT (mgg^{-1} DM TAE)	CT (mgg^{-1} DM QE)
Spring	7±0.8b*	5±0.6b	10±1b
Autumn	9±1a	6±1a	15±2a
Species			
Robinia pseudoacacia	5±1c	4±1c	13±5c
Amorpha fruticosa	9±0.6b	6±0.4b	18±2b
Morus alba	4±0.3c	2±0.3d	2±0.4d
Arbutus unedo	19±1a	15±1a	30±4a
Colutea arborescens	4±0.3c	2±0.3d	0.4±0.01d
Source of variation			
Season (A)	P<0.05	P<0.05	P<0.05
Species (B)	P<0.05	P<0.05	P<0.05
AXB (Interaction)	P<0.05	P<0.05	P<0.05

*Means followed by the same letter in the column for each independent variable did not significantly differ (P>0.05); **ns: not significant.

Table 5. Interaction between browse species and growth stage of TPH (mgg^{-1} DM TAE), TT (mgg^{-1} DM TAE), CT (mgg^{-1} DM QE) concentration in leaves and stems (mean ± SE)

Season	Species (abbrev.)	Leaves			Stems		
		TPH	TT	CT	TPH	TT	CT
Spring	R.ps.	27±1.1b	23±1.2b	101±3.5a	6±0.4e	4±0.3d	17±0.8b
	A.fr.	16±1.3d	14±1.1c	68±7.6c	10±0.7c	7±0.5c	16±1.7b
	M.al.	11±1.6e	7±0.4d	1±0.03f	4±0.3fg	2±0.2ef	0.5±0.05e
	A.un.	34±2.1a	28±1.8a	16±2.2e	16±1.0b	12±0.7b	18±2.8b
	C.ar.	7±0.3f	4±0.2d	1±0.05f	3±0.2g	1±0.2f	0.4±0.02e
Autumn	R.ps.	20±0.7c	16±0.7c	83±7.4b	4±0.3fg	3±0.2e	9±0.8c
	A.fr.	18±1.2cd	15±1.1c	40±4.1d	8±0.9d	5±0.6d	20±3.2b
	M.al.	7±0.6f	4±0.5d	1±0.1f	4±0.5fg	3±0.4e	3±0.6d
	A.un.	36±1.9a	29±1.6a	48±1.0d	22±1.4a	18±1.3a	43±2.5a
	C.ar.	8±0.5f	4±0.3d	1±0.1f	5±0.3f	3±0.3e	0.4±0.01e

*Means followed by the same letter in each column for the same independent variable did not significantly differ (P>0.05).

Discussion

The nutritive parameters of the tested species in both leaves and stems were varied related to the season. The CP contents of the leaves and stems of the investigated species during the spring season were significantly higher than those harvested in autumn. It is well known that the decrease in CP content is due to the thickness of the cell walls in the maturity stage (Blair *et al.*, 1981; Holechek *et al.*, 1989). However, this decrease was sharper in stems than in leaves, because they had higher content of CP compared to stem during both seasons (Ball *et al.*, 2001). Generally, more than the 40% of the total N (Kramer and Kozlowski, 1979) is found in leaves of the ligneous plant species, and thus they have higher concentration of CP compared to stems (Holechek *et al.*, 1989; Mero and Uden, 1997).

The higher CP content of the leguminous species is expected as they usually contain 25 to 50% more CP than non-leguminous ones (Cheeke, 1992; Jamala *et al.*, 2013). Papachristou and Papanastasis (1994) investigated 10 woody fodder species and found similar CP contents for *R. pseudoacacia* and *C. arborscens*. Crude protein is a good indicator to determine the quality of forage (Amiri and Mohamed Shariff, 2012). According to the results of CP in the tested species it could exceed the demands of small ruminants for maintenance and lactation except *A. unedo* that cover only for maintenance (NRC, 1981, 1985). Despite the fact that *M. alba* had lower CP content than the leguminous species, this content was relatively high and thus *M. alba* was suggested by Dwi Yulistiani *et al.* (2015) as a supplement source to provide fermentable energy and fermentable protein to rice straw diet.

On the other hand, NDF and ADL contents were increased due to maturation in both leaves and stems. *A. unedo* and *R. pseudoacacia* had the higher increment in their NDF and ADL content in autumn. On the contrary, *M. alba* and *C. arborescens* had the lower one. The average chemical composition of the three leguminous species is consistent to the findings of Papachristou and Papanastasis (1994), while Khazaal *et al.* (1993) reported similar results for *A. unedo*. Moreover, these results are in agreement with other researchers worked on seasonal effect to the nutritive value of fodder species (Kamalak, 2006; Kokten *et al.*, 2012). In addition, NDF and ADL contents in stems were higher than in leaves. Similar results have been found by Griffin and Jung (1983) and Pootaeng-on *et al.* (2015). Season had a different effect on the tested species due to the high variability in the nutrient content among the browse species. This variability could be attributed to the species variability owing to factors such as plant part, morphological differences within the same species, season harvesting etc. (Solomon 2001; Beyene, 2009). These results are in agreement with findings for browse species reported by Shenkute *et al.* (2012).

The lower IVDMD during autumn was expected as the proportion of structural carbohydrates in the plant increases with maturity. Thus, the cell walls contents and the digestibility decreased (Van Soest, 1982). Moreover, the *in vitro* digestibility of the stems was lower compared to the leaves, as they had higher NDF and ADL content than leaves. Stems were less digestible compared to the leaves at advanced maturity as lignin deposition in the cell walls increased with maturation (McDonald *et al.*, 1995). Additionally, leaves are 20% more digestible than stems (Cordesse *et al.*, 1991). *Morus alba* seems to be a valuable species as a feed for small ruminants as it had relatively high CP content and IVDMD. The results of the present study confirm previous reports conducted on the same species (Parissi, 2003; Dwi Yulistiani *et al.*, 2015). On the other hand, *A. unedo* has very low IVDMD. Conversely, Hajer *et. al.* (2004) referred relatively higher IVDMD (64%) in spring for the same species, while the chemical composition was similar to the present study.

The concentration of TPH, TT and CT in leaves was not different between spring and autumn. Conversely, the concentration of TPH, TT and CT in stems was increased from spring to autumn. Contrasting results have been reported about the concentration of phenols in relation to the maturity stage. In a previous study, Glyphis and Puttick (1988) referred that the concentration of phenols increased or remained stable as leaves became more mature. *A. unedo* had the higher concentration of all species in both leaves and stems followed by the two leguminous species *R. pseudoacacia* and *A. fruticosa*. These species could be defined as tanniniferous species because of their high CT compared to the other species (Parissi *et al.*, 2014). The presence of secondary metabolites as tannins in these species probably contributed to their higher ADL content in leaves. The high concentration of condensed tannins in the legumes species is measured as lignin (pseudo lignin) (Mueller-Harvey and McAllan, 1992) and leads to its overestimation. Koukoura and Nastis (1994) have recorded similar results for the total phenols concentration of all the above tested species as a whole plant sample. Leaves had higher concentration of TPH, TT and CT than stems in both maturity stages. Similarly, Salawu *et al.* (1997) reported that the leguminous species *Calliandra calothyrsus* had more CT in leaves than in stems (Mendes Guimarães-Beelen, 2006). This high concentration of the phenolic compounds, mainly condensed tannins, may reduce the availability of nitrogen that is necessary for rumen microbial growth cause of the formation of tannin-protein complexes (Molan *et al.*, 2001; Osuga *et al.*, 2005). It is known that high concentration of condensed tannins in leaves decreased the nutrient utilization and digestibility and N retention (Kumar and Vaithiyanathan, 1990; Mueller-Harvey and McAllan, 1992, Silanikove *et al.*, 1996; Piluzza *et al.*, 2013).

It is consider that a concentration of this compound at 3-4% dry weight can have positive effect on digestion (Robbins *et al.*, 1998). Vitti *et al.* (2005) reported that tannins concentration between 2-4 % of DM is beneficial for digestion and a concentration above 5% resulted harmful for metabolism. However, these limits in tannin concentration must not generalized while others points of view related to plant may be taken into account. There was a decrease in leaves CT concentration from spring to autumn in the two leguminous species *R. pseudoacacia* and *A. fruticosa*. Similar results have been found for *O. viciifolia* (Berard *et al.*, 2011) and *Acacia* sp. (Salem, 2005). According to Singh *et al.* (1997) and Joseph *et al.* (1998) enzymes responsible for the production of CT in these species probably have decreased their activity as the plant matures. In addition, Skogsmyr and Fagerstrom (1992) reported that plants use more the soluble carbohydrates for their growth and seed production, than for tannins production, which has higher metabolic cost. However, *C. arborescens* and *M. alba* had very low tannins concentration both in spring and autumn. On the contrary, higher concentration in *A. unedo* was observed with the advanced maturation of the leaves. Kokten *et al.* (2012) reported similar findings for other Mediterranean shrub species.

The results of the present study provide evidence than the concentration of phenols through maturity is species-dependent. Several factors may affect the concentration of CT in leaves. The quantity of CT to the foliage could vary according to the genotype (Baldwin *et al.*, 1987) and their concentration and extractability changes with season and plant maturity (Hagerman, 1988; McMahon *et al.*, 2000; Salem, 2005). Moreover, differences in the analytical procedures for tannins determination could lead to large variations in tannin concentration (Makkar, 2003b). Inconsistencies could be also due to sampling and climatic effect on foliage growth and plant nutrient accumulation (Salem, 2011). All the above factors alone or in combination had an effect on the nutritive value of the foliage as a livestock feed. The knowledge of tannins concentration due to seasonality could be useful for rangeland management practices to improve the ruminants' welfare and production. Thus, fodder species as *R. pseudoacacia*, *A. fruticosa* (Papachristou *et al*., 1999) and *M. alba* (Parissi, 2003) could be integrated in silvopastoral systems or used as supplement feed during autumn.

Conclusions

According to the findings of this study, the impact of maturation on the type and concentration of phenols and tannins is species-dependent. Based on their chemical composition, IVDMD and the concentration of condensed tannins, the investigated browse species could be ranked as follows: *M. alba* > *R. pseudoacacia* > *C. arborescens* > *A. fruticosa* > *A. unedo*. The nutritive value of stems was generally lower compared to leaves. In particular, *M. alba* seems to be a valuable feed resource for ruminants. The decreased tannins concentration in leguminous species *R. pseudoacacia* and *A. fruticosa* during autumn can result useful for utilize these species as valuable feed for livestock. However, this perspective needs to be confirmed also by an *in vivo* digestibility trial.

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