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Production and Nutritional Quality of low elevation zone Grasslands and Kermes Oak Shrublands (*Quercus coccifera* L.) in the South-East Mediterranean Basin

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Abstract: Problem statement: The objective of this study was to evaluate the production and nutrition quality of grasslands and kermes oak shrublands in Preveza Prefecture, northwest Greece. **Approach:** The study involved the collection of herbage and browse samples along a grazing period, from March to October for two consecutive years. In consequence, browse samples were manually separated to leaves and twigs. The three forage components (herbage, kermes oak leaves and twigs) were analyzed for crude protein, Neutral-Detergent Fiber (NDF), Acid-Detergent Fiber (ADF), lignin and *In Vitro* Dry Matter Digestibility (IVDMD). After the 2 years of observation, we concluded that kermes oak browse, especially the leaves, had CP content adequate to fulfill the nutrient requirements of grazing goats throughout the grazing period. **Results:** However, this browse is characterized by a very low IVDMD during summer, which may be the result of high lignin content and the presence of anti-nutritional factors such as phenols and tannins. The month of harvest strongly affected the production and nutritive value of forage components, firstly by giving a significant positive correlation among average monthly temperature, production, cell wall contents and secondly, a negative correlation among average monthly rainfall, IVDMD and lignin content. **Conclusion:** In closure, kermes oak shrublands of the lower zone constitute an important alimentary source providing adequate amounts of browse in order to maintain the nutrient requirements for grazing goats during the summer, when the herbage is not desirable for consumption from sheep and cattle.

Keywords: Herbage, kermes oak, nutritional quality, Greece

INTRODUCTION

In Greece, rangelands considered to be important feed resources for extensive Livestock Farming, extended mainly in the rural and Less Favorable Areas (LFAs) of the country. However, in these areas extensive livestock farming plays a key role to both rangeland ecosystem sustainability and in the viability of the primary sector (Chatzitheodoridis *et al*., 2008).

Grasslands and kermes oak shrublands are two of the main range types in Epirus which support an important grazing livestock population throughout the year.

The most important factor that affects grassland production and nutrition quality is the climate (Buxton, 1996). Greece has a typical Mediterranean climate characterized by mild and rainy winters, relatively warm and dry summers and, generally, extended periods of sunshine during the year. As a result, grasslands present a highly seasonal growth pattern involving two minimum peaks, a bigger one in the summer and a smaller one in the winter. These minimums are virtual feed gaps creating serious problems in animal production, especially in the arid and semi-arid zones (Papanastasis *et al*., 2008).

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Woody species, such as kermes oak (*Quercus coccifera* L.), are well adapted in Mediterranean climate particularly to summer drought (Tsiouvaras, 1987) and are considered as common components of Mediterranean vegetation growing alone or in a mixture with other sclerophyllous evergreen shrubs. Previous studies indicated that both kermes oak browse production and nutritive value are affected by environmental conditions and vary widely among species, varieties and seasons (Koukoura, 1988; Papanastasis *et al*., 2008; Platis and Papanastasis, 2003; Tsiouvaras, 1987). In general, kermes oak browse is rich in cell content, lignin and tannins but poor concerning energy, crude protein and digestibility (Kamalak *et al*., 2004; Koukoura, 1988; Papachristou and Nastis, 1994; Papanastasis *et al.*, 2008). The low digestibility levels are associated to intense presence of secondary compounds, especially lignin and tannins.

There is some evidence that shrublands are more productive than grasslands in comparable environments, but such case is based on the shrubland structure and more specific to shrub density and height (Papanastasis *et al*., 2008). In addition, producers developed the impression that kermes oak browse offer to livestock a relatively higher nutrition quality feed than herbage, during the dry season when herbaceous plants complete their biological cycle and therefore, their nutritional quality is considerably low. For this reason, it is estimated that kermes oak browse can fulfill the nutritional needs during summer and winter feed gaps, if evergreen (Papanastasis *et al*., 2008), or can be used as feed supplements for goats during summer (Noitsakis and Papanastasis, 1992).

Lastly, the aim of this study was to evaluate the production and nutritional quality of grasslands and kermes oak shrublands within the grazing period in order to achieve a sustainable management for these specific ecosystems of Preveza Prefecture.

MATERIALS AND METHODS

Studied area: The study was carried out in the lower altitudinal zone rangelands of Preveza Prefecture, north-western Greece, from February 2007 till October 2008.

Long term records for the studied area are provided by Hellenic National Meteorological Service (HNMS, 2009). According to these, the climate, a typical Mediterranean, is characterized by rainy, cold winters and dry, warm summers. Mean monthly temperature ranges from 8.7-26.5°C for January and July respectively. Mean annual rainfall for the period of 1976-1997 was 1084.6 mm. One automatic weather station (Onset HOBO weather station) was placed in the experimental area in order to gather data regarding the local precipitation and temperature (Fig. 1). These data showed that dry season begins at the end of April and ends at the late of August.

Sample collection: For the experiment, during February of 2007, ten sites were selected for sampling (Fig. 2) in an altitudinal zone ranging from 125-350 m. In each site, two plots of 20 m^2 each; one in grassland and one in the adjacent kermes oak shrubland, were fenced in order to avoid grazing during the experimental period. Furthermore, inside each enclosure, eight randomly selected quadrates of 0.25 m^2 were selected. Both grassland and kermes oak shrubland selected, had the same morphological appearance. The sampling period was appointed to monthly intervals according to the grazing period, lasting from March till October. In grasslands, sampling was done by cutting herbaceous vegetation using hand shears at approximately 5 cm above soil level. For kermes oak shrublands, samples obtained by cutting all new twigs. All samples were immediately placed into individual paper bags and transported to the laboratory, where, they were divided in two groups, twigs and leaves. This was necessary because goats prefer to eat leaves of deciduous woody plants such as kermes oak. Dry sample weights were recorded after drying samples for 48 h in a 55°C oven and weighed to determine dry matter production on a g m^{-2} basis. All samples were ground in a Kinematica Mill (model Polymix PX-MFC 90 D) fitted with a 1 mm screen prior analysis.

It is important to note that the dominant plant species of herbage was based on frequency of occurring above 5% in the eight quadrates equals 2 m^2, in each plot at the blooming stage, near June (Table 1).

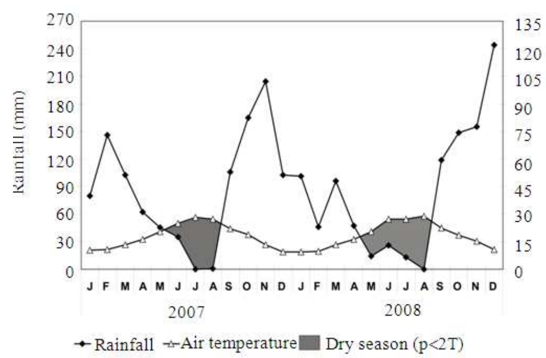

Fig. 1: Climatic diagram of the study area. Data obtained during the period January 2007 to December 2008

Table 1: Plant species with a frequency of occurring above 10% present in June

Botanical components		
Grasses	Legumes	Forbs
Agrostis canina L.	*Astragalus monspessu-lanus* L.	*Asparagus acutifolius* L.
Avena barbata ex Link	*Lotus corniculatus* L.	*Cistus incanus* L.
Avena sterilis L.	*Medicago lupulina* L.	*Convolvulus arvensis* L
Brachypodium distachyon L.	*Medicago minima* L.	*Crepis* sp.
Briza minor L.	*Trifolium repens* L.	*Galium verum* L.
Bromus maximus Desf non Gilib	*Trifolium subterraneum* L.	*Geranium* spp.
Cynodon dactylon (L.) Pers.	*Vicia angustifolia* L.	*Ranunculus repens* L.
Dactylis glomerata L.	*Vicia cracca* L.	*Stachys germanica* L.
Dasypyrum villosum (L.) Coss. and Durieu	*Vicia villosa* Roth	*Teucrium polium* L.
Hordeum crinitum (Schreb.)		
Hordeum murinum L.		
Hyparrhenia hirta (L.) Stapf		
Lolium multifloium Lam.		
Oryzopsis miliacea L. Asch. et Schweinf		
Phalaris minor Retz.		
Sorghum halepense (L.) Pers		
Stipa bromoides (L.) Dörfl		
Taeniatherum caput-medusae (L.) Nevski		

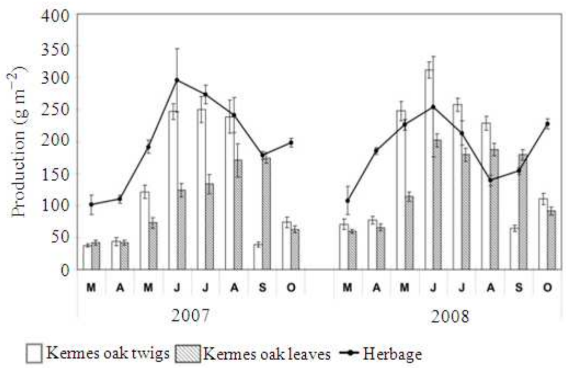

Fig. 2: Dry matter production in each forage component over the two years. Vertical bars ± SE

Chemical analyses: Each sample was analyzed for crude protein by the kjeldahl distillation method and further calculated by multiplying N by 6.25. Nitrogen was determined using a Vapodest distillation unit (model Vapodest 40 distillation system, Gerhardt). Neutral-Detergent Fiber (NDF), Acid-Detergent Fiber (ADF) and lignin were determined using Fibertec fiber analysis system (model Fibertec M6 fiber analysis system, FOSS, Denmark) according to the (Van, 1994) method. *In vitro* Dry Matter Digestibility (DMD) was determined according to the two stage technique (Tilley and Terry, 1963) using the ANKOM Daisy II-200 incubator (Ankom Technology, Fairport, NY 14450).

Statistical analyses: Statistical differences were tested using a two-way Analysis of Variance (ANOVA) following the Generalized Linear Model (GLM) procedure of SPSS version 16 for windows (Norusis, 2008). Least Square Differences (LSD) were used to determine significant differences among means when significant ANOVA results occurred. The Pearson's correlation was employed to examine relationships between nutritive quality variables, climatic variables and altitude. The relationships among altitude, climatic and nutritive quality variables along with forage production, were tested using linear regression over mean values for each forage component.

RESULTS

Forage production: After the evaluation of the chemical and statistical results, we concluded that production showed significant variations between forage components and the month of sampling (Table 2). At the beginning of the experimental period, herbage production was significant ($p<0.05$) higher than browse production (twigs and leaves). This was reversed at the summer, where browse production was higher than herbage's. However, during the second experimental year, the herbage production was decreased as a consequence of low rainfall levels compared to increased browse production (Fig. 2). Both herbage and browse production presented maximum and minimum values at June and September, respectively; while they showed similar trends with climatic variables (Table 3), chemical composition and *in vitro* DM digestibility (Table 4). However, the correlation coefficients regarding to kermes oak leaves are lower than those of herbage and kermes oak twigs. Specifically, mean monthly temperature and mean monthly rainfall were significantly related to production (Table 3).

Table 2: Results of two-way ANOVA showing degrees of freedom, F-statistic and the significance of the effects of forage component (herbage, kermes oak leaves and twigs), month and factors interactions on production and nutritive quality parameters

		Production		CP		NDF	
Effect	d.f.	F	P	F	P	F	P
Forage component (F)	2	178.438	0.000	564.758	0.000	183.012	0.000
Month (M)	7	170.257	0.000	492.164	0.000	775.954	0.000
F × M	14	30.321	0.000	204.211	0.000	123.324	0.000
		ADF		ADL		IVDMD	
Effect	d.f.	F	P	F	P	F	P
Forage component (F)	2	270.342	0.000	5607.492	0.000	4739.744	0.000
Month (M)	7	532.385	0.000	587.4041	0.000	1117.159	0.000
F × M	14	56.502	0.000	54.17771	0.000	95.05151	0.000

Table 3: Coefficients associated climatic variables* for significant variance in a stepwise multiple regression analysis of the production (as a dependent variable) for the period of March to June

Regression equation		R^2	SE
$\text{Production}_{\text{herbage}}$	= -69.93+16.861 temperature	0.640	48.54
$\text{Production}_{\text{kermes oak leaves}}$	= -133.05+20.582 temperature-0.515 rainfall	0.805	45.82
$\text{Production}_{\text{kermes oak twigs}}$	= -35.258+9.599 temperature-0.302 rainfall	0.736	27.04

Level of significance p<0.05, *: Mean monthly temperature and rainfall of month before sampling

Table 4: Correlation coefficients of production of each forage component with climatic and nutritional quality parameters

Parameter	Herbage (n = 80)	Kermes oak leaves (n = 80)	Kermes oak twigs (n = 80)
Rainfall	-0.457**	-0.441**	-0.786**
Temperature	0.582**	0.381**	0.809**
IVDMD	-0.647**	-0.548**	-0.598**
Crude protein	-0.746**	-0.274**	-0.458**
NDF	0.664**	0.574**	0.350**
ADF	0.642**	0.708**	0.629**
Lignin	0.618**	0.663**	0.493**

Level of significance: ** p<0.01

Table 5: Correlation coefficients between nutritive quality parameters of each forage component and average monthly temperature before sample harvest

Parameter	Herbage (n = 80)	Kermes oak leaves (n = 80)	Kermes oak twigs (n = 80)
IVDMD	-0.852**	-0.735**	-0.529**
Crude protein	-0.863**	-0.396**	-0.440**
NDF	0.870**	0.457**	0.473**
ADF	0.872**	0.576**	0.674**
Lignin	0.869**	0.566**	0.435**

Level of significance: ** p<0.01

Chemical composition: The crude protein content of all forage components was significantly different among the months of sampling. This difference is expanded to forage components within the sampling months (Table 2). The CP content regarding all forage components displayed an overall rapid decrease throughout the period from March to July followed by a relatively low concentration level during August and September and finally, a smaller but significant increase located in October (Fig. 3).

As far as kermes oak concern, during summer the leaves presented significantly (p<0.05) higher crude protein content and lower cell wall contents compared to kermes oak twigs and herbage (Fig. 3). More specific, for the two years of the experiment, the average highest and lowest CP percentage for kermes oak leaves were 14.2 and 8.4% respectively. Additionally, the CP content of kermes oak twigs was relatively low and declined after the sampling of April, with content levels falling below goats requirements.

Figure 3 shows the CP and fiber contents (NDF, ADF and lignin) of the forage components during the grazing period. It is remarkable that during the period from March to September, the CP concentration levels decreased while NDF, ADF and lignin contents increased for all forage components.

The cell wall contents (NDF, ADF and lignin) for all forage components were significantly different among the months of sampling and among forage components within the months of sampling.

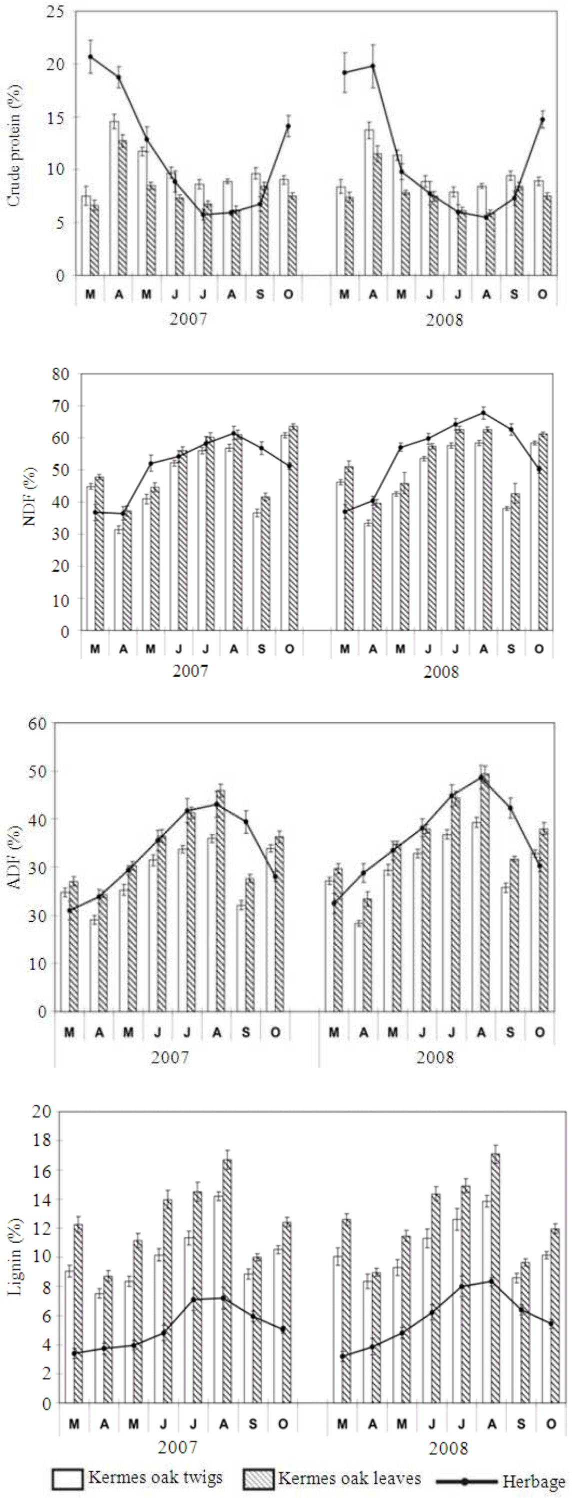

Fig. 3: Crude protein, NDF, ADF and lignin contents (%) in each forage component of the two years of study. Vertical bars ± SE

Table 6: Correlation coefficients between nutritive quality parameters of each forage component and average monthly rainfall before sample harvest

Parameter	Herbage (n = 80)	Kermes oak leaves (n = 80)	Kermes oak twigs (n = 80)
IVDMD	0.686**	0.465**	0.378**
Crude protein	0.748**	0.057	0.128
NDF	-0.649**	0.023	-0.018
ADF	-0.727**	-0.228**	-0.381**
Lignin	-0.592**	-0.358**	-0.282**

Level of significance: ** p<0.01

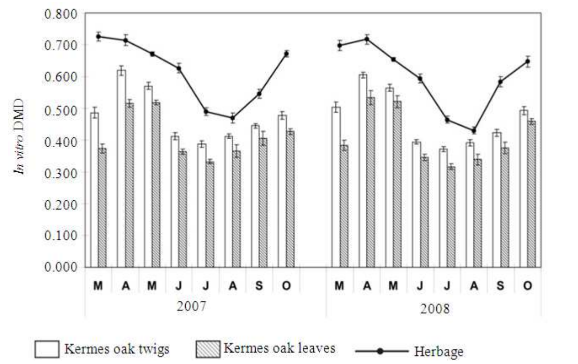

Fig. 4: *In vitro* DMD of forage components from Preveza Prefecture rangelands in each year. Vertical bars ± SE

The CP content and cell wall fractions of each forage component showed similar trends with the average monthly temperature (Table 5) and the average monthly rainfall (Table 6) before the harvest of the samples. However, fiber contents displayed the opposite trend compared to the CP content during the sampling period. During summer, all forage components presented the highest lignin content, which was significantly higher in kermes oak twigs than leaves and herbage. The lignin concentration is positively associated with the plant maturity (Bertrand *et al.*, 2008; Buxton, 1996; Koukoura, 1988; Papachristou and Nastis, 1994; Tallowin and Jefferson, 1999; Tsiouvaras, 1987). The results of this study showed that for all forage components lignin content was positively related with the average monthly temperature and negatively with the average monthly rainfall. Nevertheless, it is observed that correlation coefficients values are lower in kermes oak leaves than twigs and herbage.

***In Vitro* Dry Matter Digestibility (IVDMD):** The *in vitro* DM digestibility for all forage components was significantly different among the months of sampling and among the months of forage components within the months of sampling (Fig. 4 and Table 2). This concurs with the fact that IVDMD values showed significant negatively correlation with the average monthly temperature and positively one with the average monthly rainfall (Table 5 and 6).

Table 7: Coefficients associated climatic parameters for significant variance in a stepwise multiple regression analysis of the IVDMD (as dependent variable)

Regression equation	R^2	SE
$IVDMD_{herbage}$ = 0.792 -0.010 temperature+0.001 rainfall	0.775	0.046
$IVDMD_{kermes\ oak\ leaves}$ = 0.662-0.009 temperature	0.541	0.054
$IVDMD_{kermes\ oak\ twigs}$ = 0.538-0.006 temperature	0.280	0.063

Level of significance p<0.05

Table 8: Coefficients associated chemical composition parameters for significant variance in a stepwise multiple regression analysis of the IVDMD (as dependent variable)

Regression equation	R^2	SE
$IVDMD_{herbage}$ = 0.737-0.037 lignin+0.006 CP	0.891	0.032
$IVDMD_{kermes\ oak\ leaves}$ = 0.422-0.016 lignin+0.022 CP	0.700	0.044
$IVDMD_{kermes\ oak\ twigs}$ = 0.414-0.011 lignin+0.017 CP	0.559	0.050

Level of significance P<0.05

Also, there was a significant probability (p<0.05) for a linear relationship among IVDMD, chemical composition (Table 8) and climatic parameters (Table 7) for all botanical components. Nevertheless, the highest IVDMD values in all studied forage components were observed in spring (Fig. 4). Lastly, it is important to mention that IVDMD of herbage was always significantly higher compared to kermes oak leaves and twigs.

DISCUSSION

Forage production: The variation of herbage and browse production observed in our experiment may indicate that the browse production is less affected by rainfall than herbage, or that kermes oak can utilize effectively the rainfall than grassland species.

Generally, the growing season of herbaceous plants starts with September rainfalls and ends at the mid of June, a fact that explains the values recorded for their production. Consequently, during summer the values of herbage production are very low due to dried herbaceous vegetative matter. On the other hand, kermes oak begins a new life cycle during the second half of March with the young twigs appearing at the beginning of April. Then, kermes oak shows a rapid growth which ends nearby May, followed by a period of stem hardening during summer and finally, a second, but smaller growth period appearing in the early of September. Therefore, we can say that the browse production of March reflects the secondary growth of the previous fall.

Furthermore the similarities of the herbage and kermes oak browse production related to the climatic variables, the chemical composition and IVDMD are strongly connected to the month of harvest and the influences occurred are documented in previous studies for grasslands (Bertrand *et al*., 2008; Vázquez de Aldana *et al.*, 2000) and kermes oak shrublands (Koukoura, 1988; Noitsakis and Papanastasis, 1992; Papanastasis *et al.*, 2008).

Chemical composition: The CP content in forages generally serves as a reliable measure for the nutritional quality. The CP requirements for maintenance in diets of livestock range from 77-95 g kg^{-1} for a 40 kg live weigh goat with medium level of muscular activity and a 50 kg live weigh sheep respectively (National Research Council, 1981; 1985). Hence, according to the findings of this study, herbage CP content is inadequate to meet the daily requirements of sheep during the period from June to September. On the other hand, kermes oak leaves contain sufficient CP fulfilling the nutrient requirements for goat's maintenance.

It has been stated that increasing plant maturity is associated with the great need for structural plant material leading to cellulose, hemicellulose and lignin content increase (McDonald *et al*., 2002). This is evident while the increasing DM production formatting woody stems suggesting the importance of forage management in terms of optimising the forage quality. This fact concurs with the results obtained from the experiment and with reports given for both grassland (Bertrand *et al.*, 2008; Karn *et al.*, 2006; Tallowin and Jefferson, 1999; Vázquez de Aldana *et al.*, 2000) and kermes oak shrublands (Koukoura, 1988; Papachristou and Nastis, 1994; Tsiouvaras, 1987).

The results of this study showed that for all forage components the lignin content was positively associated with the plant maturity (Bertrand *et al.*, 2008; Buxton, 1996; Koukoura, 1988; Papachristou and Nastis, 1994; Pėrez *et al*., 1998; Tallowin and Jefferson, 1999; Tsiouvaras, 1987) and the average monthly temperature, but negatively with the average monthly rainfall, which comes to an accordance with the positive correlation between the maturity stage of the plant and temperature and with the negative one between the maturity stage and rainfall. Nevertheless, the observation that correlation coefficients values are lower in kermes oak leaves than twigs and herbage may indicate that the chemical composition of kermes oak leaves is less susceptible to temperature and rainfall fluctuations than the other forage components.

***In Vitro* Dry Matter Digestibility (IVDMD):** The overall decrease of IVDMD for all forage components was associated to environmental factors and plant maturation. Van Soest (1994) stated that temperature, light and moisture accelerate the maturation process, with DMD to decrease with plant maturity (Bertrand *et al*., 2008; Karn *et al*., 2006; McDonald *et al*., 2002). These statements concur with the correlations mentioned between IVDMD and monthly temperature and monthly rainfall from present study.

The increasing average of daily temperatures combined with a relatively high spring rainfalls would have promoted the maturation process and therefore can explain the high IVDMD values in all the studied forage components (Fig. 4).

The low digestibility levels occurring at higher temperatures are due to increased lignification and the use of the cell wall content through the metabolic processes (Van, 1994). The DM lignification degree and the CP concentration in the forage affect the digestibility of forage (McDonald *et al*., 2002; Reynolds, 2000). This explains the high herbage IVDMD compared to kermes oak leaves and twigs.

CONCLUSION

After the evaluation of the results obtained from this study, we conclude that kermes oak browse, especially the kermes oak leaves, present adequate CP content to fulfill the nutrient requirements of grazing goats through the grazing period. However, this browse is characterized by a low IVDMD level during the summer, may be the result of anti-nutritional factors such as phenols and tannins. Nevertheless, kermes oak shrublands in the lower zone constitute an important alimentary source providing amounts of browse with a scope to maintain nutrient requirements for grazing goats during the summer, when the herbage is not consumable desirable for sheep and cattle. Conclusively, Preveza Prefecture rangelands illustrate a nutritional graduation level between grasslands and kermes oak shrublands. So, as rangelands extent from lowlands to uplands, it is crucial to determine the monthly variations of nutritional quality parameters in relation to above sea altitude in order to optimize animal nutrition and range management.

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