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Original Article

Nutritional Quality of Herbaceous Vegetation in a Phryganic Mediterranean Ecosystem During the Grazing Period

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Abstract

Grazing is an economically and environmentally valuable activity in phryganic Mediterranean ecosystems. The herbaceous vegetation of these ecosystems provides an essential feed source for grazing ruminants. Despite the extension and economic importance of phryganic ecosystems in Mediterranean Basin there has been relatively little scientific focus on nutritional quality of their herbaceous vegetation. The nutritional quality of herbaceous vegetation in a phryganic ecosystem was assessed for two consecutive years during the grazing period (March to July). Herbage samples were analysed for chemical composition, metabolizable energy, *in vitro* dry matter digestibility, *in vitro* neutral detergent fiber digestibility and mineral content (Ca, P, K, Mg) at monthly basis. According to the results time of harvest significantly affected crude protein, neutral detergent fiber, acid detergent fiber, lignin and the content of minerals (i.e. Ca, P, Mg, K). Mean monthly temperature found to have a great negative impact on nutritional quality. During the grazing period, the nutritional quality of herbage vegetation was strongly correlated with growing degree days. Over a threshold of about 700 growing degree days the nutritional quality of herbage dramatically decreased. Overall the current study provides crucial information for the nutritional dynamics of the herbage vegetation of the phryganic Mediterranean ecosystems that can be towards developing an efficient grazing strategy.

Keywords: chemical composition, growing degree days, digestibility, phrygana, minerals

Abbreviations: ADF, acid detergent fiber; CP, crude protein; GDD, growing degree days; IVDMD, in vitro dry matter digestibility; IVNDFD, in vitro neutral detergent fiber digestibility; ME, metabolizable energy; NDF, neutral detergent fiber

Introduction

Phrygana, known as 'tomillares' in Spain, 'garrigue' in France, 'gariga' in Italy and 'coastal sage' in California, are open, dwarf shrub communities widespread in dry areas. It is a characteristic vegetation type of the Mediterranean ecosystems that usually grows on dry, rocky and friable soils (Debazac and Mavrommatis, 1969; Kyparissis *et al.*, 1997; Cherubini *et al.*, 2003). Phrygana have adopted a peculiar strategy to withstand the summer droughtstress of the Mediterranean climate by replacing the large winter leaves by the much smaller summer ones in order to conserve water (Kypasissis and Manetas, 1993; Kyparissis *et al.*, 1997).

Phlomis fruticosa L. (Jerusalem sage), a semi-deciduous drought-hardy and flammable dwarf shrub of about 150 cm tall (Greuter *et al.*, 1986), is the dominant species of phryganic ecosystems in lower and middle altitudes of Western Greece. In these ecosystems, a rich herbaceous flora usually occurs providing a valuable feed source for extensive livestock farming systems in marginal areas that are unsuitable for agriculture use (Kandrelis, 2000).

However, *Phlomis fruticosa* is an unpalatable species to ruminant livestock. Therefore, its encroachment results in a significant reduction of grazable species and in turn decreases grazing capacity (Roukos *et al.*, 2008). On the other hand, there is a widespread concern about the negative impact of livestock

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overgrazing on phryganic ecosystems. Negative impacts of overgrazing in such ecosystems are dramatically increased when combined with burning (Papanastasis *et al*., 2002) and possibly lead to desertification (Geist and Lambin, 2004).

Several factors affect the nutritional quality of herbaceous vegetation which, in turn, directly influences the pasture ability to sustain animal production (Holechek *et al*., 2010). Climatic conditions, botanical composition, soil properties and topography may differentiate the nutritional quality of herbage, which needs to be precisely estimated in order to determine a proper management plan (Vazquez-de-Aldana *et al*., 2000; Ayan *et al*., 2006; Bovolenta *et al*., 2008; Ramirez *et al*., 2009; Holechek *et al*., 2010; Mountousis *et al*., 2011; Roukos *et al*., 2011a).

Variations in herbage nutritional quality are reflected in animal performance which is related closely to dry matter intake and nutrient digestibility (Coleman and Moore, 2003). Consequently, the risk of metabolic disorders can be increased when livestock graze low quality herbage. For example, grass tetany is related to the mineral composition of the feed (Jefferson *et al*., 2001) and occurs primarily in lactating cows, less frequently in lactating sheep and occasionally in lactating goats (Caple and West, 1992; Pugh and Baird, 2012).

Most of the studies on the nutritional quality of herbaceous vegetation in the Mediterranean Basin have been carried out in grasslands and shrublands. On the contrary, few such studies have been conducted in phryganic ecosystems which are characterized by harsher soil, special climatic and vegetation characteristics compared to the grasslands (Montalvo *et al*., 1991; Roukos *et al*., 2011b).

Greece is predominantly a mountainous country with phryganic ecosystems growing from the sea level up to the sub-alpine region in northern areas of the country (Egli, 1991). However, apart from studies on assemblages of the phryganic vegetation by Papanastasis *et al*. (2002), Bergmeier (1997) and Bergmeier & Matthäs (1996) and management (Kandrelis, 1995; Kandrelis, 2000; Roukos *et al*., 2008) no detailed research has been carried out on the effects of climatic condition on the nutritional quality of herbage yield at the phryganic ecosystems.

The aim of this study was to determine the nutritional quality characteristics of the above ground herbage biomass of a phryganic ecosystem in relation to its monthly development during the grazing period.

Materials and Methods

Study area and climate

This investigation was conducted on the foothills of Xirovouni Mountain (20.942940° longitude, 39.370576° latitude) located in northwestern Greece. The study area is a typical phryganic ecosystem of western Greece extended between shrublands and grasslands. In this intricate spatial mosaic are extended 3,150 hectares of phrygana vegetation which is be grazing of approximately 1,000 head of cattle and more than 30,000 head of sheep under extensive management. These figures are based on a live weight of 50 kg for sheep and a 200 kg for free-ranging beef cattle (with average daily growth of less than 0.5 kg).

Long-term climatic records for the study area were provided by the Hellenic National Meteorological Service (HNMS, 2009). These records indicated a typical Mediterranean climate, characterised as Csa according to Köppen (Hatzianastassiou *et al*., 2008). The average air temperatures in January and July was 8.7 °C and 26.5 °C, respectively, (considering data from 1976-1997) while the mean annual rainfall 1,084.6 mm. Soils are calcaceous with pH values ranging between 7.1 and 7.8, containing rather insufficient availability of N, P and K (Roukos *et al*., 2011b). Geologically, the plain belongs to the Ionian geotectonic zone. The basic substrates are dolomites, Viglas limestones and flisch.

Sampling methods and experimental data collection

Field data were collected during two consecutive growing seasons within 10 experimental plots of 5 m × 5 m each with western-southwestern aspects. The experimental plots were established during January and slightly different in altitude above sea level which ranged from 125 m to 476 m. Herbaceous vegetation was dominated by five grasses (*Avena sterilis*, *Dactylis glomerata*, *Melica ciliata*, *Piptatherum miliaceum*, *Stipa bromoides*), eight legumes (*Anthylis hermaniae*, *Medicago falcata*, *Medicago lupulina*, *Medicago minima*, *Trifolium arvense*, *Trifolium campestre*, *Trifolium purpureum*, *Trifolium subterraneum*) and five forbs (*Daucus guttatus*, *Plantago bellardii*, *Scabiosa* sp., *Scolymus hispanicus*, *Stachys germanica*). Hand-clipped samples were collected for five monthly intervals from the beginning of the growing period (early March) until the senescence of plant species (early July). Sampling was performed by cutting the herbaceous biomass at ground level at approximately 5 cm within eight randomly selected quadrats of 0.25 m^2 inside each experimental plot at monthly basis. Samples were immediately placed into individual paper bags, transported to the laboratory and dried for 48 h in a 55 °C oven, ground with a Kinematica mill (model Polymix PX-MFC 90D), passed through a 1 mm sieve and stored at 4 °C until further analysis.

Climatic data during the experiment were collected from two automatic weather stations (Onset HOBO weather station) installed in the experimental area (Fig. 1). For each sample per month, precipitation and temperature data were associated with the nearest weather station. Then, accumulated growing degree days (GDD) were calculated for each month of harvest using the mean-minus-base method (Pruess, 1983; Frank and Hofmann, 1989; Frank *et al*., 1998) using the formula: GDD = [(maximum daily temperature + minimum daily temperature)/2] – base temperature.

The basal growth temperature was set at 10 °C as it reflects the essential temperature for the initiate development of warm season grasses (Frank, 1996), although lower value has been reported from Unruh *et al*. (1996). Also, a lower limit of 10 °C was set for the minimum temperature. Growing day degrees were summed from 1 January of each year according to Hendrickson *et al*. (1998).

Analytical methods

Monthly herbage samples were analyzed for dry matter (DM), ash, ether extract and crude protein (CP) (methods 930.15, 942.05, 920.39 and 976.05, respectively, of the A.O.A.C., 2000).

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and sulfuric acid lignin (lignin(sa)) were determined using an ANKOM Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to procedure describes by Van Soest *et al*. (1991) and modified by Vogel *et al*. (1999) for the ANKOM

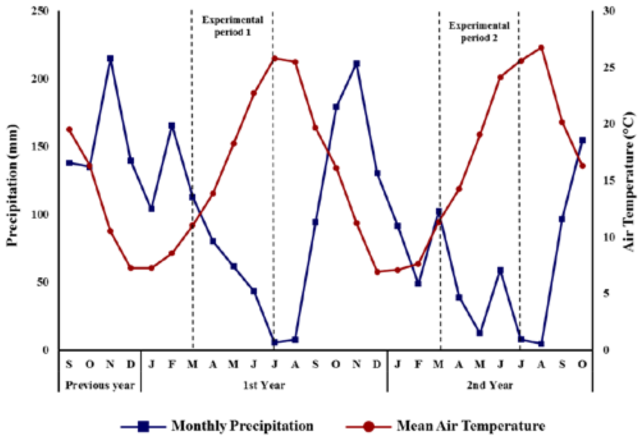

Fig. 1. Climatic diagram of the study area during the two years experimental period

system. For NDF analysis, samples were treated with heat-stable amylase (A3306, Sigma–Aldrich, St. Louis, MO, USA) without sodium sulfite in the neutral detergent (ND). NDF and ADF are expressed without residual ash. *In vitro* dry matter (DM) digestibility (IVDMD) was measured according to Vogel *et al.* (1999) using an ANKOM DaisyII incubator (ANKOM Technology). Rumen fluid was obtained from eight non-lactating Holstein cows fed according to their nutritional requirements. The gross energy of each herbage sample was measured using an adiabatic bomb calorimeter (model IKA C5000, IKA Works Inc., Wilmington, NC, USA). Post-digestion analyses were completed on the undigested residue to determine the NDF concentration using the above-described methods, and on the gross energy of the residue using a bomb calorimeter. *In vitro* neutral detergent fiber (NDF) digestibility (IVNDFD) was calculated according to Hall and Mertens (2008), as: IVNDFD (g/kg NDF) = (1 − [post-digestion dry weight following ND wash/pre-digestion dry weight of NDF]). Additionally, digestible energy was determined using the following formula: DE (MJ/kg DM) = (pre-digestion gross energy − [gross energy in residue × (1− IVDMD)]). Metabolisable energy (ME) was calculated using an equation adapted from NRC (2006): ME = DE × 0.82.

Concentrations of K, Ca, Mg and P in the herbage samples were assessed in triplicate by oxidizing each subsample with a 2:1 nitric/perchloric acid mixture. In separate aliquots, Ca and K were determined by flame photometry, P by spectrophotometric methods (Khalil and Manan, 1990) and Mg by atomic absorption spectrophotometry (AOAC, 1999; method 968.08) (model PERKIN ELMER/AA800, PerkinElmer Inc., San Jose, CA, USA).

The risk of grass tetany was evaluated using an equation adapted from Kemp and Hart (1957) as: grass tetany index (GTI) = $K^+/(Ca^{2+} + Mg^{2+})$, with each element expressed in mmolc/kg DM (mg/kg DM × 1000 × valence/atomic weight).

Statistical analyses

One-way analysis of variance (ANOVA) was carried out to determine the effect of time of harvest on chemical composition, mineral contents and nutrient digestibility of herbage samples. The normality of the distribution was determined through the Shapiro-Wilk's test. Since the samples had a normal distribution, the one-way ANOVA with LSD's post hoc test was used. Differences were considered statistically significant at the $P < 0.05$ level. In addition, the Pearson's correlation was employed to examine relationships between nutritive quality variables, climatic variables and altitude (Steel and Torrie, 1980). The relationship between growing degree days and nutritive quality variables were tested using regression analysis. All analyses were conducted using IBM SPSS Statistics v. 21.0.0 (New York, USA) software.

Results and Discussion

CP and cell wall contents

Month of harvest significantly ($P<0.001$) influenced the CP, ether extract and cell wall contents of herbage (Table 1) during the grazing period. The CP and ash content had a progressive decrease throughout the grazing period, while cell wall contents increased dramatically from March to July. Correlation analysis revealed that CP and ash contents are negatively ($P<0.01$) associated with mean monthly air temperature ($r=-0.940$ and $r=-0.667$, respectively). On the contrary, cell wall contents are positively correlated with mean monthly air temperature (Table 2).

The CP content is a reliable measure of herbage nutritional quality (Ganskoop and Bohnert, 2001) as affects dry matter intake through the microbial activity in rumen (Merkel *et al.*, 1999). High dietary CP levels are positively associated with the degradation of protein in the rumen and have been shown to decrease the efficiency of nitrogen utilization for milk production (Broderick, 2003; Hristov *et al.*, 2004).

The adequate CP requirement threshold for maintenance of livestock range from 82 g/kg for growing beef cattle (with a live weight of 300 kg and an average daily gain of 0.22 kg) to 95 g/kg for sheep (with a live weight of 50 kg) (NRC, 1985; 1996). Thus, except July, the CP levels in herbage were sufficient to meet the daily requirements of sheep and free-ranging beef cattle and can be considered to be of high nutritional quality for grazing livestock.

The CP content of forage reflects the climatic conditions during plant growth and development (Holechek *et al.*, 2010) and is markedly decreased with forage plant maturity (Perez-Corona *et al.*, 1998; Ramirez *et al.*, 2004; Mountousis *et al.*, 2008; Roukos *et al.*, 2010; Mountousis *et al.*, 2011; Acar *et al.*, 2016). Additionally, ether extract tended to increase from March to May and decreased afterwards till July. This pattern is in agreement with SCA (1990), and Mountousis *et al.* (2008).

The proportion of NDF, ADF and lignin considerably increased in herbage throughout the grazing period as it is associated with the plant maturation, a result also found by Perez-Corona *et al.* (1998), Roukos *et al.* (2010), Mountousis *et al.* (2011) and Acar *et al.* (2016).

Moreover, the voluntary dry matter intake is limited by NDF concentration higher than 600 g/kg due to rumen fill (Mertens, 1994; Allen, 1996). The NDF content of herbage exceed the above mentioned threshold only on July, when the highest ADF and lignin contents during the grazing season were observed. However, during June NDF content was close to the above threshold and can be regarded as high level.

The highest lignin content was observed at the last two months of the grazing period (June and July), probably as a consequence of the significant increase of air temperature during this period of the year.

Table 1.Effect of harvest date on crude protein (CP), ether extract (EE) and cell wall concentrations of herbaceous vegetation during grazing period

Parameter	March	April	May	June	July	SEM	Sig.
CP (g/kg DM)	206a	199a	155b	107c	73d	3.8	***
EE (g/kg DM)	15ab	15a	17a	16a	14b	0.6	*
NDF (g/kg DM)	334e	409d	561c	591b	641a	13.4	***
ADF (g/kg DM)	236e	292d	333c	386b	435a	10.2	***
Lignin(sa)(g/kg DM)	33d	44c	44c	64b	71a	2.2	***

Within a row, means with different letters differ at P<0.05.
SEM = standard error of the mean; Sig = significant level
*P < 0.05; **P < 0.01; ***P < 0.001; NS = not significant

Table 2.Correlation coefficients between chemical composition parameters of herbaceous vegetation and climatic parameters during the grazing period

Parameter	Mean montly air temperature	Monthly Precipitation
CP (g.kd DM)	-0.940**	0.634**
EE (g/kg DM)	0.086	-0.152
NDF (g/kg DM)	0.863**	-0.676**
ADF (g/kg DM)	0.916**	-0.624**
Lignin (g/kg DM)	0.882**	-0.539**
Ash(g/kg DM)	-0.657**	0.448**
ME (MJ/kg DM)	-0.596**	0.554**
IVDMD	-0.887**	0.510**
IVNDFD	-0.896**	0.534**
P (g/kg DM)	-0.440**	0.182
K (g/kg DM)	-0.476**	0.307**
Ca (g/kg DM)	-0.171	0.006
Mg (g/kg DM)	-0.294**	0.223*
GrassTetanyIndex	-0.149	0.193

* P<0.05; ** P<0.01.

Mineral composition and grass tetany index

In general, herbage had Ca, P, K, Mg and ash contents that were significantly (P<0.05) different between time of harvest (Table 3). On the other hand the GT index did not significantly differ among the harvest dates. Mineral and ash concentrations diminished from the second month of harvest (April) onwards showing their lowest values in July. Similar trends in mineral and ash concentration were found by Mountousis *et al.* (2006; 2008) and can be caused by the increased air temperature (Bertrand *et al.*, 2008) and its negative impact on plant mineral intake from the soil (Wang *et al.*, 2006). Also, the reduction in mineral and ash content during plant growth has been attributed to the increased dry matter yield due to dilution of minerals in plant tissue (Pelletier *et al.*, 2006).

Minerals are essential to livestock for both growth and milk production (Suttle, 2010). In general, mineral content in herbage depends chiefly on its botanical composition, soil and climatic conditions during growing season (Holechek *et al.*, 2010; Suttle, 2010; Roukos *et al.*, 2011a).

Herbaceous vegetation is generally satisfactory sources of Ca for grazing livestock, particularly when they contain leguminous species, thus calcium deprivation rarely affects grazing livestock (Suttle, 2010). Ewes with a 50 kg live weight and beef cattle with a 200 kg live weight require about 2 g of Ca/kg DM (NRC, 1985) and 1.2 g of Ca/kg DM (NRC, 1996), respectively. It seems that herbage during the grazing period had sufficient Ca levels to meet these requirements.

Phosphorus maintenance requirements for beef cattle and sheep are 1.7 g/kg DM (NRC, 1996) and 1.6 g/kg DM (NRC, 1985) of their diet, respectively. According to the results, the herbage had sufficient P levels to meet the maintenance requirements of grazing livestock. Roukos *et al.* (2011a) reported higher values of Ca and P in herbage collected from grasslands in adjacent area. On the contrary, Ganskoop and Bohnert (2001) and Ramirez *et al.* (2009) found inadequate levels of P in forages to meet maintenance requirements of sheep and cattle in grasslands of arid environments of USA and Mexico, respectively.

Potassium is most associated with nerve and muscle excitability as well as water and acid–base balance. This element must be evaluated in relation to Mg as high K levels in diet may cause Mg deficiency due to interactions between these minerals in absorbance (Suttle, 2010). All herbage samples during the grazing period had adequate K levels to meet the optimum maintenance requirements for beef cattle (6 g/kg DM; NRC, 1996) and sheep (5 g/kg DM; NRC, 1985). Herbage biomass with sufficient K content to meet the maintenance requirements for beef cattle and sheep was also found by Ganskoop and Bohnert (2001), Ramirez *et al.* (2009) in grasslands of northern Great Basin and Mexico, respectively, and by Roukos *et al.* (2011a) in adjacent grasslands.

The optimum Mg requirements for sheep and beef cattle are 1.2 g/kg DM (NRC, 1985) and 1.0 g/kg DM (NRC, 1996) respectively. The results from this study suggest that in these phryganic ecosystemsonly during March, April and June the herbaceous vegetation is on the borderline to meet the sheep requirements in Mg, while the Mg levels are insufficient to meet the beef cattle requirements throughout the grazing period. Thus, it can be assumed that there is an increased risk of Mg deficiency to occur in grazing livestock, a result also found by Roukos *et al.* (2011a). Conversely, Ramirez *et al.* (2009) reported that 13 grasses in Mexico had marginal sufficient levels of Mg to meet growing beef cattle requirements.

Minson (1990) stated that seasonal variation of Mg content within species are relative small and Mg concentration in herbage is affected by its botanical composition and climatic conditions (Mountousis *et al.* 2008; Suttle, 2010; Roukos *et al.*, 2011a). Leguminous species have higher Ca and Mg content than grasses (Minson, 1990; Roukos *et al.*, 2011a). Calcium and Mg content decreased from April to July and may reflect a lower legume contribution in vegetation composition (Suttle, 2010; Roukos *et al.*, 2011a).

Magnesium is important for the metabolic stability of animals and must be evaluated in relation to K and Ca (NRC, 2005). A K/(Ca + Mg) ratio in excess of 2.2 (milliequivalent cation basis) can accelerate grass tetany (Kemp and Hart, 1957; Grunes and Welch, 1989; Goff and Horst, 1997), especially for cows in early lactation (Jefferson *et al.*, 2001).

The results of this study demonstrated that magnesium concentration was low in spring when potassium concentration was high. It seems that air temperature plays a role in the mineral content of herbage (Table 3). The rapid increase of air temperature during spring and the relative high K level in herbage compared to Ca and Mg, increases the risk of grass tetany for grazing livestock. This suggestion concurs with Roukos *et al.* (2011a). Conclusively, the highest GTI value for the herbage (GTI ≤ 1.39) was much lower than the threshold value of 2.2 throughout the grazing period.

Nutrient digestibility

In vitro DM digestibility, IVNDFD and ME significantly varied ($P<0.05$) among harvest months (Table 4). All nutrient digestibility parameters had a progressive decrease during the grazing period which negatively correlated with air temperature (Table 2). It seems that CP content influenced positively nutrient digestibility parameters because when CP decreased, all parameters also decreased (Table 2). Conversely, cell wall contents negatively affected nutrient digestibility parameters. These results concur with the results of Ramirez *et al.* (2009), Mountousis *et al.* (2011) and Roukos *et al.* (2011a).

Moreover, a positive effect of mean monthly precipitation on nutrient digestibility parameters was revealed (Table 2). Precipitation influenced chemical composition and thus affected the nutrient digestibility parameters. Similar results also found by Ganskoop and Bohnert (2001), Mountousis *et al.* (2008) and Roukos *et al.* (2011a).

The IVDMD values in herbage samples ranged from 0.521 in July to 0.726 in March. These values concur with those reported by Perez-Corona *et al.* (1998), Vazquez de Aldana *et al.* (2000), Mountousis *et al.* (2008; 2011) and Roukos *et al.* (2011a).

Although IVNDFD is an empirical measurement of fiber fermentability by rumen microbes (Hall and Mertens, 2012), it might be a better indicator of DMI than NDF digestibility *in vivo* and, thus, is an important parameter of forage quality (Oba and Allen, 1999). The IVNDFD negatively correlated with cell wall content (Table 5) and decreased significantly throughout the grazing period (Table 4). *In vitro* NDF digestibility values ranged from 0.683 in March to 0.430 in July and are in agreement with those reported by Oba and Allen (1999), NRC (2001), Roukos *et al.* (2011). The low digestibility of NDF during the last two months of the grazing period, when lignin(sa) content was the highest, can have a dramatic impact on dietary energy content and DM intake (Oba and Allen, 1999).According to McDonald *et al.* (2002), lignin may bind cellulose and cell wall protein in strong chemical bonds, which makes these compounds undigestible.

The estimated ME values of the herbage ranged from 7.29 MJ/kg to 10.95 MJ/kg DM (Table 4). Similar resultshave been reported by Mountousis *et al.* (2011) and Roukos *et al.* (2011). The ME contents were sufficient to meet the maintenance requirement of 50 kg live weight sheep (8.37 MJ/kg DM; NRC, 1985) and 200 kg live weight free ranging beef cattle (8.07 MJ/kg DM; NRC, 1996).

Relationship between growing degree days and nutritional quality parameters

Regression analyses quantified relationships between CP, NDF, lignin, IVDMD, IVNDFD and ME values of herbage and growing degree days (GDD) as independent variable (Fig. 2). The mathematical and statistical parameters of the regression analysis are given in Table 6.

All nutritional digestibility parameters were strongly associated with growing degree days. Moreover, the accuracy of the equation to predict IVDMD and CP content from growing degree days was very high (Adj. $R^2>0.90$) and the accuracy of IVNDFD, ME, NDF and lignin contents was satisfactory (Adj. $R^2>0.77$). According to the regression analysis, an increase of 100 growing degree days resulted in a

Table 3. Effect of time of harvest on mineral concentrations, ash content and grass tetany index (GT index) of herbaceous vegetation during the growing period

Parameter	March	April	May	June	July	SEM	Sig.
Ca (g/kg DM)	3.88a	4.19a	3.72a	3.43bc	2.88c	0.381	*
P (g/kg DM)	3.10a	3.05a	2.12b	2.39b	2.14b	0.232	***
K (g/kg DM)	14.3a	12.8ab	11.9bc	11.2c	9.3d	0.824	***
Mg (g/kg DM)	1.09a	1.03a	0.81b	1.02a	0.83b	0.092	**
GT index (mmol$_c$/kg DM)	1.389a	1.147a	1.258a	1.241a	1.239a	0.132	NS
Ash (g/kg DM)	113.7a	99.4b	82.5c	68.3d	56.2c	4.75	***

Within a row, means with different letters differ at $P<0.05$.
SEM = standard error of the mean; Sig = significant level
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant

Table 4.Nutrient and energy digestibility of herbage vegetation in a prhyganic ecosystem during the grazing period

Parameter	March	April	May	June	July	SEM	Sig.
IVDMD	0.726a	0.710b	0.671c	0.626d	0.521e	0.005	***
IVNDFD	0.683a	0.675a	0.665a	0.479b	0.430c	0.009	***
ME (MJ/kg)	10.95a	10.68a	9.87b	8.27c	7.29d	0.125	***
GE (MJ/Kg)	18.60b	18.76bc	18.84bc	18.89b	19.46a	0.095	***

Within a row, means with different letters differ at $P<0.05$.
M = mean; SEM = standard error of the mean; Sig = significant level
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant

Table 5.Correlation coefficients between nutritive quality parameters and chemical composition of herbage vegetation during the grazing period

Parameter	IVDMD	NDF Digestibility	Metabolisable energy
CP	0.863**	0.867**	0.881**
NDF	-0.802**	-0.754**	-0.827**
ADF	-0.866**	-0.826**	-0.844**
Lignin	-0.833**	-0.888**	-0.836**

* P<0.05; ** P<0.01.

Table 6. Coefficients associated with growing degree days (GDD) for significant[a] variance regression analysis of crude protein (CP, g/kg DM), neutral detergent fiber (NDF, g/kg DM), lignin (g/kg DM), *in vitro* dry matter digestibility (IVDMD), *in vitro* NDF digestibility (IVNDFD) and metabolizable energy (ME, MJ/kg DM) as dependent variables during grazing period

		Regression equation	Adjusted R^2	SE
CP	=	0,000063 $(GDD)^2$ – 0.205 (GDD) + 219	0.912	16.2
NDF	=	0.197 ln(GDD) + 163	0.849	10.3
Lignin	=	-0,000015 $(GDD)^2$ – 0.052 (GDD) + 32	0.778	7.3
IVDMD	=	-0,000189× (GDD) + 0.738	0.911	0.02
IVNDFD	=	-0,00027× (GDD) + 0.711	0.825	0.04
ME	=	0.0000015 $(GDD)^2$ – 0,0053 (GDD) + 11	0.858	0.57

[a] Significant at the 0.05 probability level

decrease of 0.019 for IVDMD and 0.027 for IVNDFD. These results indicate that growing degree days is an important factor affecting the nutritive value of herbaceous vegetation in a phryganic ecosystem.

Studies have provided evidence that GDD is a useful indicator of forage maturity worldwide, since temperature is the most significant climatic variable affecting the rate of plant growth and development (Bootsma, 1984; Frank and Hofmann, 1989; Frank, 1996; Hendrickson *et al.*, 1998; Nordheim-Viken *et al.*, 2009; Peterson *et al.*, 2010; White *et al.*, 2012). Based on the results of this study, it is recommended that high nutritional quality herbage obtained during the period from March to May when GDD did not exceed the value of 700. It seems that GDD values over 700 have a markedly negative impact on nutritional quality parameters of the herbaceous forage of a phryganic ecosystem.

Conclusions

This study provided evidence that harvest month significantly affected the nutritional quality of the herbaceous vegetation in phryganic ecosystems. During the grazing period, herbage biomass leaded to a decline in overall nutritional quality.

In the first part of grazing period, phryganic ecosystem herbage can be considered to be of high nutritional quality as it meets the livestock requirements. In this period, GDD value did not exceed the threshold of about 700, over which the nutritional quality of herbage dramatically decreased. In the last part of the grazing period, a supplementation of protein, energy and minerals in ruminant diets is needed.

The prediction equations, based on the GDD, provided satisfactory accuracy of IVDMD, IVNDFD and CP, NDF and ME contents of herbage. These equations can be utilized in similar phryganic ecosystems in the Mediterranean region.

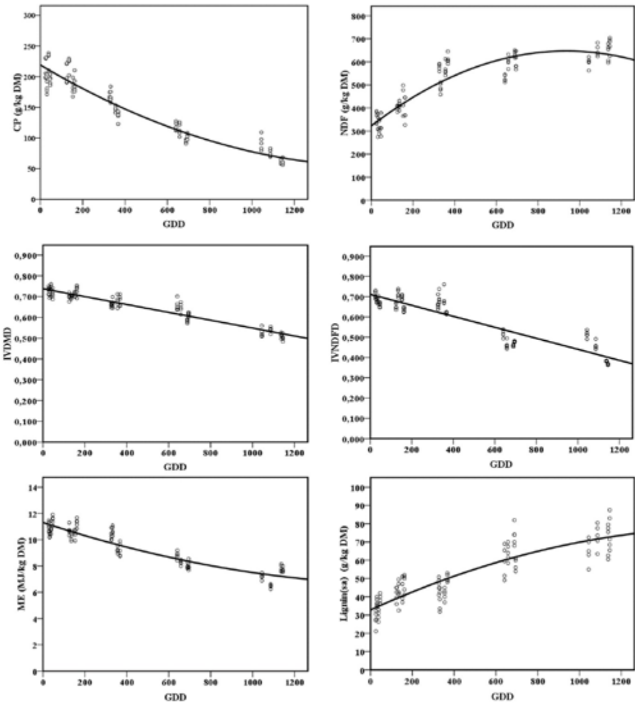

Fig. 2. Relationships between growing degree days (GDD) and CP, NDF, lignin and ME content, IVDMD and IVNDFD. The symbol (○) indicates the observed values

Nevertheless, in order to improve the accuracy of the prediction equations based on the GDD, which is a parameter relatively independent of the climatic variability, further research is required.

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