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SEASONAL AND ALTITUDINAL VARIATIONS IN NUTRITIONAL QUALITY OF KERMES OAK (*QUERCUS COCCIFERA* L.) IN NORTHWEST GREECE AND EXTENSIVE GOAT FARMING

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Abstract

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The growth and nutritional quality of kermes oak (*Quercus coccifera* L.) are affected by climatic environment and stage of maturity. The study aimed to determine changes in the nutritional quality of kermes oak components (i.e., twigs and leaves) in shrublands across a mountainside, where climatic conditions change with altitude above sea level, in northwest Greece. The study involved monthly collection of forage samples during March–October from 15 experimental plots located in three altitudinal zones (lower, middle, and upper) over two consecutive years. Samples were manually separated into the two shrub components and analyzed for crude protein (CP), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom), lignin(sa), *in vitro* dry matter (DM) digestibility (IVDMD), *in vitro* neutral detergent fiber digestibility (IVNDFD), digestible energy (DE) and minerals (i.e., Ca, P). Altitudinal zone, in terms of existing climatic conditions, strongly affected the nutritive value of leaves and twigs. Indeed, altitude above sea level was positively correlated with mean monthly precipitation and negatively correlated with mean monthly air temperature, which, in turn, strongly influenced CP, IVDMD, IVNDFD, fiber contents, DE, and mineral contents. The Ca:P ratio exceeded the animal functional disorder threshold, which posed a hazard.

Key words: kermes oak, chemical composition, digestibility, Greece

Introduction

Kermes oak (*Quercus coccifera L.*) is a sclerophyllous evergreen shrub that is well adapted to the Mediterranean climate (Tsiouvaras, 1987). Its shrublands are one of the main range types in Greece, stretching from lowlands to mountainous areas where extensive goat farming plays a key role in cost-effective production of safe and high-quality animal-origin products, and rangeland sustainability (Chatzitheodoridis et al., 2007; Papachristou et al., 2005).

Indeed, goat farming is the most productive use of regions dominated by shrublands thanks to the goats' ability to survive and the economic viability of free-grazing in shrublands in which other ruminants would find survival difficult (Papanastasis et al., 2008). Furthermore, grazing goats rely on kermes oak browse for 32.5–61.4% of their total annual feed requirements (Papachristou and Nastis, 1993).

These findings led us to study the browse nutritional quality and sustainable management of kermes oak shrublands in Greece (Tsiouvaras, 1987; Koukoura, 1988; Papachristou et al., 2005; Papanastasis et al., 2008).

In northwest Greece, along mountainsides, topography (in terms of altitude above sea level) influences local and regional microclimates by changing the patterns of climatic variables, such as precipitation and temperature, which influences soil properties (Roukos et al., 2011b) and forage production (Mountousis et al.; 2006; Roukos et al., 2011a). Climate–topography interactions create a spatial of rangeland mosaic, resulting in variations in nutritive value for grazing animals. Although the effect of altitudinal zone on forage

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nutritional quality has been studied extensively (Mountousis et al., 2006; Roukos et al., 2011a), little is known about the effect of altitudinal zone on the nutritional quality of kermes oak browse.

This study aims to estimate the nutritional quality of the kermes oak in relation to the main shrub components along a mountainside divided into three altitudinal zones.

Materials and Methods

Study area

The study was conducted on Xirovouni Mountain (longitude: 20.942940°, latitude: 94 39.370576°) in Epirus, northwest Greece, over two years (February 2007–October 2008). The study area is distinguished by its diverse landscape with natural alternations.

Within the intricate landscape of Xirovouni Mountain, 7220 hectares of natural shrubland pastures are maintained under extensive management, and support about 8500 native-breed goats with a live weight of about 40 kg.

Long-term climatic records for the lower altitudinal zone of the study area were provided by the Hellenic National Meteorological Service (HNMS, 2015). These records indicated a typical Mediterranean climate characterized by cold, rainy winters and warm, dry summers. Mean monthly temperatures in January and July were 8.7 and 26.5°C, respectively, and mean annual rainfall between 1976 and 1997 was 1085 mm. For our study, three automated weather stations (Onset HOBO weather station, Onset Computer Corporation, MA, USA) were installed (one per altitudinal zone) to record local precipitation and temperature fluctuations among the zones throughout the study period (Figure 1).

Sampling method

During February 2007, 15 sites with western–southwestern aspects, representing typical kermes oak shrubland conditions in northwest Greece, were selected for monthly sampling. Three zones were differentiated based on altitude above sea level: upper (above 1001 m), middle (501–1000 m) and lower (0–500 m). In each, five experimental plots of 20 m^2, with at least six kermes oak shrubs of the same age and morphological appearance but with different altitudes above sea level, were fenced to exclude grazing. Throughout the two years, hand-clipped samples were collected from each plot at monthly intervals, from March–October, by cutting all new twigs within four randomly selected quadrats of 0.25 m^2 inside each enclosure. Samples were immediately placed into individual paper bags, transported to the laboratory and manually separated into leaves and twigs. Next, the samples were air dried for 96 h at 25°C, ground using a Kinematica mill (model Polymix PX-MFC 90D) through a 0.5 mm sieve and stored at 4°C for further analysis.

Analytical methods

The N content of each sample was determined using a Kjeldatherm KB 8 S-BS digestion unit and Vapodest 40 distillation unit (Gerhardt, GmbH & Co. KG, Germany) using AOAC (1995) method 984.13. Neutral detergent fiber (aNDFom) was determined using an ANKOM Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methods proposed by Van Soest et al. (1991) and modified by Vogel et al. (1999) for the ANKOM system. The aNDFom content was analyzed using heat-stable amylase (A3306, Sigma–Aldrich, St. Louis, MO, USA) without sodium sulfite in the neutral detergent (ND). Acid detergent fiber (ADFom) was determined according to the AOAC's (1997) method 973.18. aNDFom and ADFom are expressed without residual ash. Lignin (sa) was determined by the sulfuric acid procedure (AOAC, 1997; method 973.18), and ash was determined as the gravimetric residue after heating to 550°C for 8 h. *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 native-breed goats 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 aNDFom 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 equation:

DE (MJ/kg DM) = (pre-digestion gross energy − [gross energy in residue × (1− IVDMD)]).

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

Statistical analyses

The data were analyzed based on a split plot design with altitudinal zones (n = 3) as main plots and time of harvest

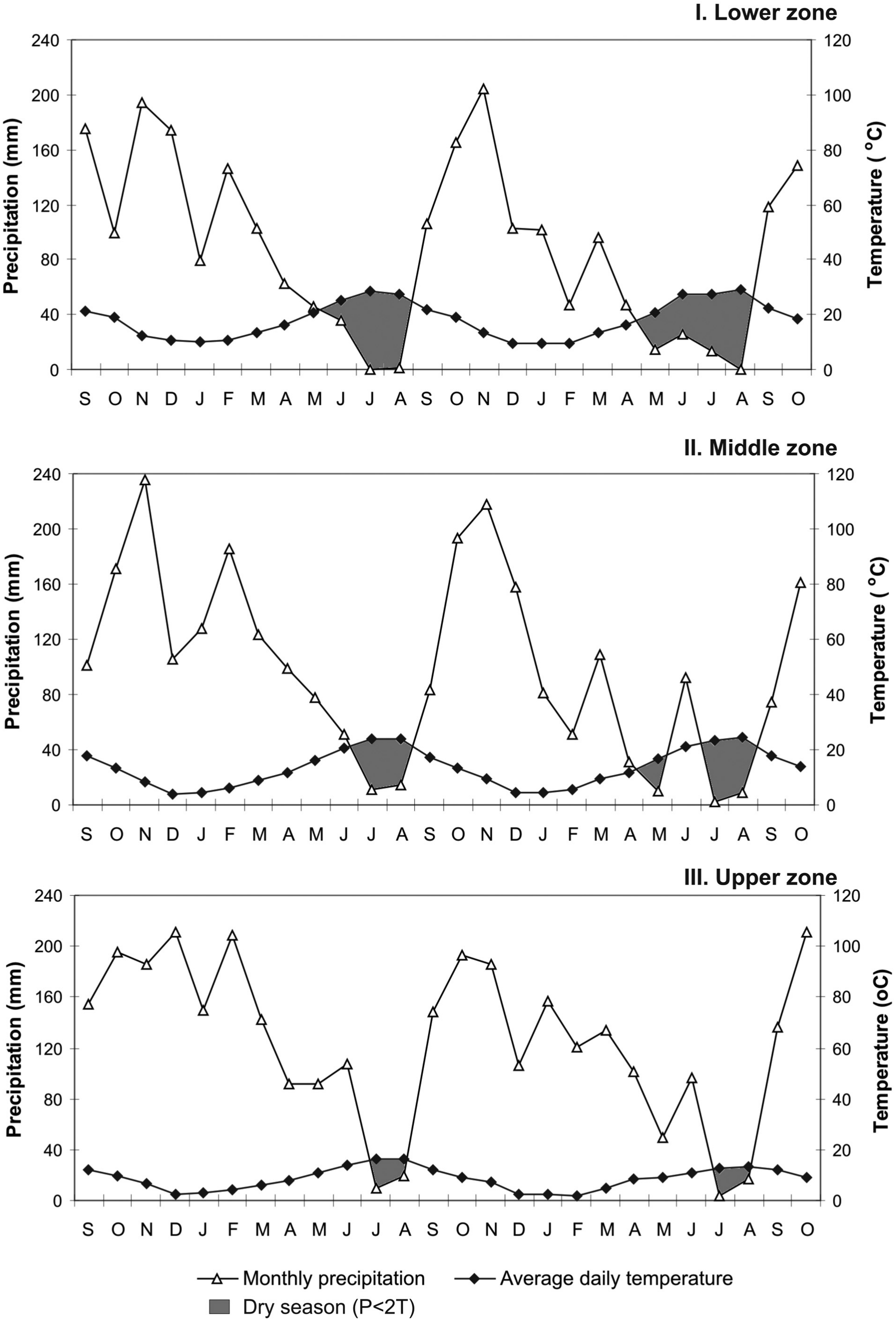

Fig. 1. Climatic diagrams from the three automated weather stations installed (one per altitudinal zone) to record local precipitation and temperature fluctuations among the zones throughout the study period

(n = 8) as sub-plots (Snedecor and Cochran, 1980). The experimental plots and year of harvest were considered random effects. The data were analyzed using SPSS software (IBM Statistical Package for the Social Sciences, Version 20, 2011). The interaction altitudinal zone × month of harvest was significant ($p < 0.05$); thus, analyses of variance were conducted among altitudinal zones and among altitudinal zones within month of harvest. Significant mean differences were detected using least square differences (Steel and Torrie, 1980). Pearson's correlations were calculated to examine interactions between nutritive variables, chemical composition, monthly precipitation and air temperature. Additionally, correlations among variables, altitude above sea level, air temperature and nutritional quality were assessed using linear regression. Differences were considered significant at the $p < 0.05$ level.

Results and Discussion

Climatic conditions

The altitudinal zones differed in temperature and precipitation in the two experimental years (Figure 1). Mean monthly precipitation and air temperature were correlated with altitude above sea level ($r = 0.318$; $p < 0.01$ and $r = -0.501$; $p < 0.01$, respectively).

According to average values over the two-years, both mean annual precipitation and mean monthly rainfall were higher in the upper than in the middle and lower zones. However, mean monthly air temperature was higher in the lower than in the other two zones.

The results demonstrated that the highest temperatures occurred in the lower zone, and the highest rainfall in the upper zone. Thus, each altitudinal zone has its own climate, which likely affected growth of the kermes oak shrub.

Crude protein and cell wall contents

Over the two-years, analyses of crude protein (CP) and fiber contents revealed effects of altitudinal zone and month of harvest (Tables 1 and 2). CP was lowest and fiber highest in leaves and twigs during summer, as supported by the negative correlation between CP content and mean monthly air temperature ($p < 0.01$, Table 5). In spring, CP content was higher than that identified by Tsiouvaras (1987) and Ataşoğlu et al. (2010) for kermes oak shrublands of Greece and western Turkey, respectively. We assume that the more favorable climatic conditions, especially greater precipitation, in Xirovouni Mountain compared to the areas of the other studies contributed to this result. Additionally, due to the relatively high precipitation during the growing season (Figure 1), the CP contents of leaves and twigs did not significantly differ ($p > 0.05$) among the altitudinal zones.

The CP content of forage is not only an important indicator of forage quality, but also a result of climatic conditions during plant growth and development (Buxton, 1996). Studies have shown that kermes oak growth is affected by climatic conditions (Tsiouvaras, 1987; Koukoura, 1988; Ataşoğlu et al., 2010). In the study area, the presence of a one-month time-shift in kermes oak growth from lower to middle and middle to upper zones was found. As a result, differences occurred in CP and fiber contents among altitudinal zones within the month of harvest (Tsiouvaras, 1987; Koukoura, 1988; Tolunay et al., 2009; Ataşoğlu et al., 2010).

Low CP in kermes oak can reduce browse digestibility, feed intake and livestock growth (Van Soest, 1994). Intake declines sharply when forage contains <7% CP (McDonald et al., 2010). Based on the study results, the CP content of kermes oak leaves was over this threshold.

Dietary CP requirements for maintenance of free-range goats with a live weight of 40 kg reaches 93 g (NRC, 1981), corresponding to 77 g/kg of dry-matter intake. Average CP levels in the leaves were sufficient to meet the daily requirements of goats throughout the grazing period. On the other hand, the CP content of kermes oak twigs is sufficient to meet the goats' daily requirements only during spring in all altitudinal zones.

Interestingly, the fiber contents of kermes oak leaves and twigs were higher in the lower zone ($p<0.05$; Tables 1 and 2). The fiber contents of each kermes oak browse component showed similar trends with altitude above sea level (Tables 1 and 2).

The fiber content of forages varies depending on environment and stage of maturity (Buxton, 1996). High temperatures increase fiber fractions and enhance lignin synthesis (Wilson et al., 1991), reducing dry-matter digestibility (DMD) (Buxton, 1996). ADF content over 18% in forage dry matter can reduce the feed intake of goats (Santini et al., 1992). The average ADF content of KO leaves and twigs was over this limit throughout the grazing period, so it seems that goats that feed only on kermes oak browse cannot maximize their dry-matter intake.

As temperature increases, the proportion of NDF, ADF and lignin in the kermes oak browse components also increase. This pattern is well revealed by the positive correlations between fiber contents and means monthly air temperature (Table 5), and was also found by Tsiouvaras (1987), Koukoura (1988), Tolunay et al. (2009) and Ataşoğlu et al. (2010).

Dietary fiber also plays a pivotal role in goat production through its influence in and interaction with nutrient intake and digestion (Lu et al., 2005), and in milk-fat content (Schmidely et al., 1999). Optimum NDF concentrations

Table 1
Monthly and altitudinal zone means of crude protein, neutral detergent fiber, acid detergent fiber, acid detergent lignin, Ca, P and Ca:P ration in kermes oak leaves (g/kg DM)

Altitudinal Zone	Month of harvest								Mean	SEM	Sig.
	M	A	M	J	J	A	S	O			
CP											
Lower	105	145	116	94	85	88	96	91	103	2.8	***
Middle	112	147	134	110	78	75	95	92	105	4.4	***
Upper	103	109	143	131	112	90	79	84	106	4.3	***
Mean	107	134	131	112	92	84	90	89			
SEM	5.9	5.1	4.6	5.0	2.6	1.7	2.3	2.1			
Sig.	NS	***	***	***	***	***	***	***			
NDFom											
Lower	332	397	403	501	549	583	597	456	477	13.2	***
Middle	350	351	464	522	567	576	588	518	492	8.8	***
Upper	317	320	349	483	541	552	549	531	455	17.2	***
Mean	333	356	405	502	552	570	578	502			
SEM	8.7	9.1	14.6	14.2	17.2	13.8	14.1	11.2			
Sig.	*	***	***	*	NS	NS	**	***			
ADFom											
Lower	205	206	292	347	376	410	400	266	312	8.1	***
Middle	253	212	274	302	350	363	370	253	297	10.8	***
Upper	260	276	258	275	324	330	339	326	298	9.9	***
Mean	239	231	275	305	350	368	370	282			
SEM	6.8	8.6	7.4	6.3	10.3	11.8	14.3	9.7			
Sig.	***	***	***	***	***	***	***	***			
ADL											
Lower	95	86	103	130	134	143	125	86	113	3.7	***
Middle	93	79	87	111	119	133	132	84	104	4.4	***
Upper	92	90	85	102	115	118	121	116	114	4.8	***
Mean	93	85	92	115	120	131	126	95			
SEM	4.2	3.6	4.3	6.2	5.1	6.3	5.5	4.1			
Sig.	NS	*	***	***	**	***	NS	***			
Ca											
Lower	2.4	2.3	1.9	1.8	1.6	1.6	2.0	2.3	2.0	0.08	***
Middle	2.3	2.0	1.7	1.8	1.7	1.8	1.5	1.8	1.8	0.06	***
Upper	1.7	1.4	1.5	1.3	1.2	1.2	1.7	2.0	1.6	0.07	***
Mean	2.4	2.3	1.9	1.8	1.6	1.6	1.9	2.3			
SEM	0.07	0.08	0.07	0.06	0.05	0.06	0.07	0.08			
Sig.	***	***	***	***	***	***	***	***			
P											
Lower	2.1	2.4	1.6	1.0	0.9	0.8	0.7	1.4	1.4	0.11	***
Middle	1.6	1.7	2.0	1.9	1.4	1.5	1.1	1.6	1.6	0.09	***
Upper	1.8	2.2	2.1	2.6	2.0	1.4	1.2	1.1	1.8	0.10	***
Mean	1.8	2.1	1.9	1.8	1.4	1.2	1.0	1.4			
SEM	0.07	0.08	0.10	0.12	0.08	0.07	0.06	0.09			
Sig.	***	***	***	***	***	***	***	***			
Ca:P ratio											
Lower	1.1	1.0	1.2	1.8	1.8	2.6	2.9	1.7	1.8	0.15	***
Middle	1.4	1.2	0.8	0.9	1.2	0.9	1.3	1.1	1.1	0.07	***
Upper	0.9	0.6	0.7	0.5	0.5	1.1	1.5	1.7	0.9	0.08	***
Mean	1.1	0.9	0.9	1.1	1.2	1.5	1.9	1.5			
SEM	0.06	0.06	0.07	0.11	0.12	0.18	0.23	0.14			
Sig.	***	***	***	***	***	***	***	***			

$* p < 0.05$ $**p < 0.01$ $***p < 0.001$ NS – Not Significant

Table 2
Monthly and altitudinal zone means of crude protein, neutral detergent fiber, acid detergent fiber, acid detergent lignin, Ca, P and Ca:P ration in kermes oak twigs (g/kg DM)

Altitudinal Zone	Month of harvest								Mean	SEM	Sig.
	M	A	M	J	J	A	S	O			
CP											
Lower	92	126	82	74	64	61	75	85	83	2.9	***
Middle	111	120	87	76	63	60	73	75	84	3.0	***
Upper	96	86	100	80	71	64	61	62	78	4.5	***
Mean	100	111	90	77	66	62	70	74			
SEM	3.4	5.0	6.1	2.6	1.9	1.8	2.2	2.3			
Sig.	***	***	*	NS	***	NS	***	***			
NDF											
Lower	406	444	466	640	686	697	709	501	568	11.3	***
Middle	487	421	514	585	623	626	627	580	558	11.4	***
Upper	373	372	419	518	571	585	584	573	499	16.7	***
Mean	422	412	466	581	627	636	640	551			
SEM	10.9	12.4	14.2	14.8	13.6	13.2	14.4	12.8			
Sig.	***	***	***	***	***	***	***	***			
ADF											
Lower	273	278	351	425	447	485	482	325	383	8.9	***
Middle	309	275	339	375	421	464	466	314	370	11.3	***
Upper	290	318	302	358	390	397	417	406	360	8.8	***
Mean	291	290	331	386	419	449	455	348			
SEM	8.2	8.8	8.9	8.3	12.4	11.9	10.0	7.7			
Sig.	***	***	***	***	***	***	***	***			
ADL											
Lower	144	89	134	167	188	198	194	98	152	3.0	***
Middle	139	104	129	154	177	189	198	110	150	7.7	***
Upper	125	127	113	141	157	172	166	163	146	5.8	***
Mean	136	107	125	154	174	186	186	124			
SEM	6.6	5.2	7.3	6.6	4.5	4.8	6.1	4.7			
Sig.	*	***	*	**	***	***	***	***			
Ca											
Lower	3.4	3.3	3.4	3.6	3.3	3.1	3.4	4.0	3.4	0.13	***
Middle	3.3	3.5	3.4	2.7	2.8	2.9	3.0	4.1	3.2	0.14	***
Upper	3.1	2.1	2.6	2.3	2.1	2.2	2.4	2.7	2.4	0.10	***
Mean	3.3	3.0	3.1	2.9	2.7	2.7	2.9	3.6			
SEM	0.11	0.10	0.13	0.14	0.09	0.12	0.13	0.15			
Sig.	NS	***	***	***	***	***	***	***			
P											
Lower	2.3	2.6	1.8	1.1	0.9	0.8	0.8	1.9	1.5	0.09	***
Middle	2.0	1.9	2.3	2.7	2.4	2.0	2.1	2.2	2.2	0.12	***
Upper	1.9	2.3	2.7	2.9	2.5	2.0	1.6	2.1	2.3	0.12	***
Mean	2.1	2.3	2.3	2.2	1.9	1.6	1.5	2.1			
SEM	0.09	0.11	0.14	0.15	0.13	0.09	0.08	0.09			
Sig.	***	***	***	***	***	***	***	*			
Ca:P ratio											
Lower	1.5	1.3	2.0	3.4	3.7	3.8	4.6	2.1	2.8	0.32	***
Middle	1.7	1.9	1.5	1.0	1.2	1.5	1.4	1.9	1.5	0.08	***
Upper	1.6	0.9	1.0	0.8	0.9	1.1	1.5	1.3	1.1	0.07	***
Mean	1.6	1.4	1.5	1.7	1.9	2.1	2.5	1.8			
SEM	0.09	0.07	0.11	0.24	0.27	0.19	0.33	0.11			
Sig.	NS	***	***	***	***	***	***	***			

* $p < 0.05$ **$p < 0.01$ ***$p < 0.001$ NS – Not Significant

Table 3
Monthly and altitudinal zone means of digestible energy (DE), IVDMD and IVNDFD in kermes oak leaves

Altitudinal Zone	Month of harvest								Mean	SEM	Sig.
	M	A	M	J	J	A	S	O			
DE (MJ/kg DM)											
Lower	9.94	12.51	11.85	10.12	8.91	7.88	7.78	11.46	10.05	0.201	***
Middle	9.81	13.04	12.22	10.34	9.18	7.55	7.43	11.28	10.10	0.135	***
Upper	10.33	10.89	13.69	12.98	10.77	9.56	7.88	7.97	10.51	0.133	***
Mean	10.02	12.14	12.58	11.15	9.61	8.33	7.70	10.24			
SEM	0.146	0.175	0.154	0.177	0.164	0.133	0.152	0.174			
Sig.	**	**	***	**	**	***	*	**			
IVDMD											
Lower	0.54	0.64	0.59	0.40	0.38	0.40	0.42	0.48	0.49	0.009	***
Middle	0.55	0.58	0.60	0.51	0.39	0.39	0.40	0.43	0.49	0.015	***
Upper	0.57	0.57	0.62	0.56	0.45	0.42	0.42	0.39	0.50	0.011	***
Mean	0.55	0.60	0.60	0.49	0.41	0.40	0.41	0.43			
SEM	0.012	0.011	0.011	0.019	0.010	0.026	0.010	0.009			
Sig.	NS	***	NS	***	***	NS	NS	***			
IVNDFD											
Lower	0.43	0.44	0.36	0.28	0.23	0.21	0.22	0.45	0.33	0.006	***
Middle	0.43	0.45	0.36	0.28	0.24	0.22	0.22	0.46	0.33	0.012	***
Upper	0.42	0.39	0.45	0.42	0.36	0.27	0.20	0.23	0.34	0.011	***
Mean	0.43	0.43	0.39	0.32	0.28	0.23	0.22	0.38			
SEM	0.009	0.007	0.009	0.009	0.008	0.012	0.011	0.010			
Sig.	NS	***	***	***	***	***	NS	***			

$* p < 0.05$ $**p < 0.01$ $***p < 0.001$ NS – Not Significant

range from 150–200 g NDF/kg DM for fattening ruminants to 700–750 g NDF/kg DM for mature beef cows (Buxton, 1996). For high-producing lactating dairy goats, 180–200 g ADF/kg DM or 410 g NDF/kg DM is nutritionally adequate (Lu et al., 2005). Additionally, dietary NDF concentrations higher than 600 g/kg limit DM intake due to rumen fill (Mertens, 1994).

In our study, aNDFom concentrations in twigs were close to, or greater than, this threshold during summer, although it seems that goats do not prefer to browse kermes oak twigs during this period (Papanastasis et al., 2008).

Mineral composition

The altitudinal zone and month of harvest had a strong impact ($p < 0.05$) on the Ca and P contents and Ca:P ratio of the browse (Tables 1 and 2). Generally, Ca and P concentrations in the browse correlated ($p < 0.01$) with altitude above sea level negatively and positively, respectively (Table 5). The Ca concentration in the leaves ranged from 1.2–2.4 g/kg, which was lower than in the twigs. Calcium is absorbed as Ca^{++} cations and showed greater concentration in the twigs (Epstein and Bloom, 2005).

The P contents in leaves and twigs decreased from March–September and were lowest during September, when Ca:P ratios reached 2.9 in leaves and 2.1 in twigs. The lowest P concentration of leaves was in the lower zone (0.09 g/kg DM). Similar to the results for Ca, twigs had the lowest P concentration at 0.80 g/kg DM (Table 3). The Ca:P ratio was higher in leaves than in twigs (Tables 1 and 2), and tended ($p < 0.05$) to be lower in the upper than in the lower and middle zones. Tables 1 and 2 show that leaves had higher Ca and P concentrations than twigs, which led to higher Ca:P ratios.

Two of the most abundant mineral elements for plant and animal growth are Calcium and phosphorous. Their availability in forage is important due to their roles in the metabolic functions in livestock (Underwood and Suttle, 1999).

Minimum Ca requirements for maintenance for 40 kg live-weight goats are 2.31 g/kg DM (NRC, 1981), although lower values were proposed by Meschy (2000). Our results suggest that Ca levels in the leaves would be insufficient to meet animal requirements.

Phosphorus is an essential component for both plant and animal growth. The estimated maintenance requirements for goats are 1.49 g/kg DM (NRC, 1981). Based on

Table 4
Monthly and altitudinal zone means of digestible energy (DE), IVDMD and IVNDFD in kermes oak twigs

Altitudinal Zone	Month of harvest								Mean	SEM	Sig.
	M	A	M	J	J	A	S	O			
DE (MJ/kg DM)											
Lower	8.9	11.7	10.5	8.1	6.5	5.8	6.5	10.4	8.6	0.14	***
Middle	9.0	11.6	10.3	7.8	6.4	6.0	6.2	10.7	8.5	0.10	***
Upper	9.3	9.9	12.2	10.9	8.4	6.9	6.4	6.4	8.8	0.11	***
Mean	9.1	11.1	11.0	8.9	7.1	6.2	6.4	9.2			
SEM	0.19	0.21	0.15	0.11	0.12	0.08	0.11	0.12			
Sig.	NS	***	***	***	***	***	NS	***			
IVDMD											
Lower	0.37	0.53	0.51	0.35	0.32	0.36	0.37	0.44	0.40	0.008	***
Middle	0.42	0.48	0.50	0.37	0.35	0.36	0.35	0.40	0.40	0.011	***
Upper	0.39	0.42	0.43	0.42	0.39	0.35	0.34	0.34	0.38	0.013	***
Mean	0.39	0.48	0.48	0.38	0.35	0.36	0.35	0.39			
SEM	0.009	0.015	0.013	0.010	0.008	0.009	0.006	0.008			
Sig.	***	***	***	***	***	NS	**	***			
IVNDFD											
Lower	0.35	0.37	0.25	0.15	0.12	0.10	0.10	0.37	0.23	0.006	***
Middle	0.34	0.36	0.27	0.16	0.13	0.12	0.13	0.34	0.23	0.012	***
Upper	0.31	0.30	0.35	0.29	0.20	0.17	0.15	0.13	0.24	0.008	***
Mean	0.33	0.34	0.29	0.20	0.15	0.13	0.13	0.28			
SEM	0.007	0.007	0.010	0.010	0.009	0.012	0.012	0.011			
Sig.	***	***	***	***	***	***	***	***			

* $p < 0.05$ **$p < 0.01$ ***$p < 0.001$ NS – Not Significant

Table 5
Correlation coefficients between nutritive quality parameters for each kermes oak component and climatic variables (precipitation and air temperature)

	Leaves		Twigs	
	Precipitation	Air Temperature	Precipitation	Air Temperature
Precipitation		–0.729**		–0.729**
Air temperature	–0.729**		–0.729**	
CP (g/kg)	0.327**	–0.553**	0.422**	–0.590**
aNDFom (g/kg)	–0.662**	0.820**	–0.674**	0.853**
ADFom (g/kg)	–0.577**	0.722**	–0.631**	0.787**
Lignin(sa) (g/kg)	–0.578**	0.678**	–0.562**	0.622**
IVDMD (g/kg DM)	0.461**	–0.717**	0.299**	–0.429**
IVNDFD (g/kg NDF)	0.621**	–0.667**	0.626**	–0.695**
DE (MJ/kg DM)	0.409**	–0.572**	0.475**	–0.620**
Ca (g/kg DM)	0.134*	-0.027	–0.052	0.203**
P (g/kg DM)	0.379**	–0.656**	0.349**	–0.522**
Ca:P Ratio	–0.324**	0.583**	–0.381**	0.573**

* $p < 0.05$ **$p < 0.01$

the results in Tables 1 and 2, kermes oak twigs fulfill the abovementioned nutrient requirements during the study period, but the P levels of leaves did not exceed the requirement threshold.

The P content of the leaves met the goats' nutrient requirements for maintenance only during the first two months of kermes oak primary growth and during October, when secondary growth occurs (Papanastasis et al., 2008). Throughout the study period, the P content in twigs was sufficient for the goats' maintenance requirements in all altitudinal zones.

Absorption and utilization of Ca and P depend on the Ca:P ratio in the diet and the presence of adequate amounts of vitamin D (Underwood and Suttle, 1999; NRC, 2005), which is important in cellular function and metabolic diseases related to Ca and P absorption. The optimum Ca:P ratio to reduce functional disorders ranges from 1:1 to 2:1 (Underwood and Suttle, 1999). The average Ca:P ratio of leaves in the lower zone was below the optimal range. Therefore, the lack of browse available in the Preveza Prefecture kermes oak shrublands may cause metabolic disorders in livestock.

The higher Ca content in the kermes oak browse of the lower zone is likely due to better soil properties and higher content of available Ca in soil solution, as proposed by Sebaté et al. (1995).

Seasonal variations in the mineral content of shrubs has been attributed to dilution of minerals in the shrub biomass during growth, and subsequently to refining and resorption of minerals from the plants in autumn (Pugnaire and Chapin, 1993) and the mobilization of Ca and P in the plant tissue due to the physiological functions of development (Sebate et al., 1995).

Nutrient digestibility

In vitro DM digestibility, IVNDFD and DE varied ($P < 0.01$) among altitudinal zones and month of harvest in both leaves and twigs (Tables 3 and 4). Additionally, the variation in month of harvest differed in the lower, middle and upper zones, as indicated by the interaction between month of harvest and altitudinal zones for IVNDFD and DE (Tables 3 and 4). The IVDMD of leaves was higher ($p < 0.05$) in the upper than in the middle and lower zones (Table 3). Furthermore, the IVDMD, IVNDFD and DE values of leaves and twigs were negatively correlated with the mean monthly air temperature (Table 5).

Regression analyses quantified the relationships between the IVDMD, IVNDFD and DE values of each kermes oak browse component (i.e., leaves, twigs), and the mean monthly air temperature and altitude above sea level were independent variables (Table 6). For kermes oak leaves, the mean monthly air temperature was associated with IVDMD and IVNDFD. Indeed, a 1°C rise in air temperature resulted in a decrease of 0.01 for IVDMD and IVNDFD during the primary growth period (March–July).

The DE values of the kermes oak components ranged from 5.80–13.69 MJ/kg DM. Additionally, DE values differed among altitudinal zones (Table 2), and DE values of leaves and twigs were positively and negatively correlated with monthly precipitation and mean monthly temperature, respectively (Table 4).

The recommended daily intake of DE for goats with an average live weight of 40 kg and moderate muscular activity amounts to 10.25 MJ/kg DM (NRC, 1981), although higher recommended levels have been reported (Vilena and Pfister, 1990). The DE content of kermes oak leaves and twigs exceeded this threshold for only three months of the grazing period (March–June).

Leaves generally had higher IVDMD values than twigs. This can be explained by the higher concentration of lignin in twigs compared with leaves, as shown by Wilson et al. (1993).

The overall decrease of IVDMD for kermes oak browse components was associated with climatic conditions and stage of maturity. Van Soest (1994) stated that air temperature accelerates the maturation process, with DMD decreasing with plant maturity (Koukoura, 1988; McDonald et al., 2010). These statements concur with the correlations mentioned between IVDMD and monthly temperature from the present study.

The low digestibility levels occurring at higher temperatures are due to increased lignification and the use of cell wall content through metabolic processes (Van Soest, 1994). The DM lignification degree and CP concentration in the browse affect its digestibility, as DM intake linearly decreases with the increasing NDF content (McDonald et al., 2010). This explains the high kermes oak leaves' high IVDMD compared to twigs.

Table 6

Coefficients associated with mean monthly air temperature (Tmave) and altitude above sea level (Y) for significanta variance regression analysis of IVDMD, IVNDFD and DE (MJ/kg DM) as dependent variables during primary growth of kermes oak

Regression Equation			Adjusted R2	SE
$IVDMD_{leaves}$	=	$0.764 – 0.010\ Tm_{ave} – 6 \times 10^{-5}\ Y$	0.536	0.0584
$IVDMD_{twigs}$	=	$0.529 – 0.006\ Tm_{ave} – 6 \times 10^{-5}\ Y$	0.217	0.0557
$IVNDFD_{leaves}$	=	$0.537 – 0.011\ Tm_{ave} – 3 \times 10^{-5}\ Y$	0.671	0.0441
$IVNDFD_{twigs}$	=	$0.505 – 0.014\ Tm_{ave} – 8 \times 10^{-5}\ Y$	0.744	0.0458
DE_{leaves}	=	$11.868 – 0.082\ Tm_{ave} + 4 \times 10^{-4}\ Y$	0.324	15 614
DE_{twigs}	=	$12.294 – 0.186\ Tm_{ave} – 0.001\ Y$	0.351	14 302

a Significant at the 0.05 probability level

The lower digestibility associated with elevated temperatures is usually attributed to higher NDF concentrations. Additionally, the NDF of forage grown under higher temperatures is usually less digestible than NDF of forage grown under lower temperatures due to increased lignification (Tsiouvaras, 1987; Papachristou and Nastis, 1993; Buxton and Fales, 1994). Low digestibility of NDF can dramatically impact dietary energy content and DM intake (Oba and Allen, 1999).

In our study, both kermes oak leaves' and twigs' nutrient digestibility parameters (i.e., IVDMD, IVNDFD, DE) were negatively correlated with mean monthly air temperature ($p < 0.05$; Table 5), which was negatively correlated with altitude above sea level ($r = –0.501$; $p < 0.05$). Thus, a higher nutritive value of kermes oak browse will result from high-altitude growing sites at the same stage of maturity.

Our results concur with those of Tsiouvaras, (1987), Koukoura (1988), Nastis and Malechek (1988), Papachristou and Nastis (1993), Papanastasis et al. (2008) and Ataşoğlu et al. (2010), who found that kermes oak browse digestibility is influenced by the stage of growth, climatic conditions, soil properties and browse drying method.

In shrubs such as kermes oak, *in vitro* techniques show a trend to devaluate digestibility (Wilson et al., 1971; Van Soest, 1994) due to the presence of phenolic compounds (Rogosic et al., 2009), which have antimicrobial properties and reduce the enzymatic action (Nastis and Malechek, 1988). The reduction in nutrient digestibility parameters probably arises from increased levels of lignin or anti-nutritional factors such as tannins – as supported by Nastis and Malechek (1988), Van Soest (1994) and Papanastasis et al. (2008).

Conclusion

Data demonstrate that the altitudinal gradient of a mountainside forms individual micro-climatic and soil conditions in each altitudinal zone, which directly affect kermes oak growth and nutritive value.

In Mediterranean areas, kermes oak shrublands exhibit an altitudinal gradient of the shrubs' nutritional levels, and a potential risk of metabolic disorders from Ca:P ratio absorbability caused by variable plant absorption among minerals. This indicates that Ca and P supplementation is essential. Because ruminants mainly graze in the shrublands for up to nine months, from March–November, determining monthly variations in nutritional quality parameters is crucial to minimize potential hazards of browse mineral imbalances for animal metabolism by identifying these nutritional imbalances to facilitate design of mineral supplementation plans to optimize animal performance.

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