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Mineral content of the herbage material in pastures of Mt. Varnoudas NW Greece

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Abstract. The effects of growing season and altitudinal zone on herbage production and mineral concentration (K, Na, Ca, P, Mg, Fe, Cu, Zn Mn), were studied in herbage samples harvested from pastures in north-western Greece. Herbage production was affected ($P<0.001$) by the harvest month but was not affected by altitudinal zone and "month x altitude" interaction. Harvest month had significant influence ($P<0.001$) on Mg, Fe, and Zn contents as well as ($P<0.05$) on Na content. Only the trace elements were affected ($P<0.05$) by the altitudinal zone. The "month x altitude" interaction had no affect on either macro minerals or trace element concentration. Some of the minerals studied (P, Fe, Zn and Mn) were seasonally deficient for beef cattle while Na was deficient for both cattle and sheep in all altitudinal zones. The remainder of the minerals met the nutrient requirements of grazing animals.

Key Words: Altitude, grazing season, minerals, pastures

INTRODUCTION

Rangelands cover approximately 40% of the total surface of Greece, occur in a great variety of habitats (Karagianakidou et al., 2001), and are characterized by considerable variability in species composition induced by climatic differences, adaptive advantages, biogeographical influences and insularity (Petriccione, 1995). Traditional stockfarmings of sheep and cattle utilize to a high degree the Greek native rangelands. Both herbaceous and woody plants occur in the Greek rangelands and play a significant role in grazing animals' nutrition. The importance of each plant category depends on the grazing season (Papachristou & Nastis 1993a; 1993b). Rangeland nutritional quality is affected by abiotic and biotic environmental factors including soil type, climatic regime, botanical composition and range improvement practices (Pérez–Corona et al., 1998; George et al., 2001; Ramirez, 1996).

Protein content and digestibility of dry matter have been emphasized as the main determinants of forage quality (Pérez–Corona et al., 1998). However, much less attention has been paid to minerals even though they also influence forage quality and can depress feed intake when levels are low (Provenza, 1995). The knowledge of estimated dietary mineral intake from both feed and water provides the basis for correcting deficiencies or adjusting for mineral excesses (Herd, 1997).

The purpose of this study was to evaluate herbage production and its mineral content over a six-month period, from May to October 2004 (grazing period in the Greek mountainous areas), at three altitudinal zones in pastures of openings in forests and in the sub-alpine region, where only beef cattle and sheep graze. The knowledge of herbage mineral content is judged useful for the mineral supplementation in the diet of grazing beef cattle and sheep when moving from lower to higher altitudes during summer, on the mountains in north-western Greece.

MATERIALS AND METHODS

Study area

The present study was conducted in the mountainous (openings in forests where mainly herbaceous plants occur) and sub-alpine pastures of Mt. Varnoudas in northwest Greece (lat. 40°46’ to 40°53’ N, long. 21°07’ to 21°24’E, 900-2334m above sea level), in three altitudinal zones (900-1300m, 1301-1700m and >1700m). The highest peak of Mount Varnoudas reaches 2334m. The basic geological substrate of the whole area consists of metamorphic rock textures (i.e. phyllites, gneisses and mica schists) of the Pelagonic geotectonic zone (Koroneos, 1991). The pastures of all three altitudinal zones are comprised of acid sandy – loam soils (according to the Bouyoucos (1962) hydrometer method), with a pH ranging from 4.89 to 5.65 in the lower, from 4.63 to 5.65 in middle and from 4.52 to 5.48 above 1700m. The fertility of the soil varies depending on slope, exposure, and type of vegetation. The mountain topographic slope is quite intense and, in combination with climatic conditions, which vary from zone to zone, create an impressive variation of flora from the lowest to the uppermost zone (758 taxa occur on Mt. Varnoudas (Mountousis, 2008). There are large areas covered by beech and oak forests as well as by other small trees and shrubs. The studied pastures, which were located in the openings of forests as well as in the sub-alpine region, are covered by a great diversity of herbaceous species. Water pH (springs and streams) ranged from 5.7 to 7.4 (G.C.S.L., 2005). The study area has special climatic characteristics, which differ from those of the typical Greek Mediterranean climate (Papanikolaou et al., 2002). The climate approaches the middle–European type, the major characteristics of which are a quite cold and damp winter and a rather dry summer (Papanastasis, 1982). In winter very low temperatures may be observed, sometimes reaching -23ºC, a condition considered highly unusual for the Greek climate (H.N.M.S., 2005). The rain precipitation and the air temperature for the six experimental months of 2004 are shown in Table 1.

Table 1. Rain precipitation and air temperature of the six experimental months of 2004.

PERIOD	MAY	JUN	JUL	AUG	SEP	OCT	MEAN±SD
			Rain percepetation (mm)				
2004	65,00	108,20	48,20	17,60	78,70	81,30	66,5±31,10
			Air temperature (°C)				
2004	-	19,20	22,40	21,30	11,70	14,90	17,90±4,50

Source: Florina's weather station (altitude: 651m, Lat. 40° 48′ N, Long. 21° 25′ E - H.N.M.S., 2005

On average, air temperature decreases about 0,6°C/100m from lower to higher region. This reduction is lower in the wintertime than in summer (Flocas, 1994)

On Mt. Varnoudas pastures, beef cattle and sheep graze at lower altitudes during spring and autumn and in the sub-alpine region during summertime (Papanikolaou et al., 2002).

Sampling and Experimental Analyses

The experimental work was conducted during the year 2004, from May to October, in three altitudinal zones (lower zone: 900-1300m, middle zone: 1301 – 1700m, upper zone: over 1700m) of Mt. Varnoudas pastures. In November 2003, twenty-four experimental cages, sized 4m x 5m, fenced with metallic net 1.5m high in order to obstruct free–range grazing, were placed in the pastures.

The most dominant herbaceous species (woody shrubs as well as herbaceous weeds were excluded as they are not edible by sheep and beef cattle) in all three altitudinal zones included in the herbage material were discovered by measuring the percentage of soil surface covering, using the phytosociological approach (Braun-Blanquet, 1964; Horvat et al., 1974). Species nomenclature follows *"Flora Europea"* (Tutin et al, 1964-1980).

Each experimental cage was divided into 36 equal plots. At the beginning of each month, from May to October, herbage biomass was collected from 6 of the 36 equal plots in each cage, using a $0.015m^2$ quadrate. The herbage samples (herbaceous plants edible by sheep and beef cattle) were clipped in situ at 2 cm above the soils' surface using hand sickles (Odum, 1971). Woody plants (i.e. *Juniperus sp*., *Vaccinium myrtillus* L., *Bruckenthalia spiculifolia* (Salisb.) Reichenb *Sambucus nigra* L. etc) and herbaceous weeds were not included in the samples. The harvested forage samples (six quadrates per plot per month) were handled carefully to avoid soil contamination. The collected samples from each cage were stored in paper bags and weighed instantly in the field for fresh weight determination. At the laboratory all samples were oven-dried at 68°C until reaching steady weight. Chemical composition of herbage biomass has been reported elsewhere (Mountousis et al., 2006).

Samples were analyzed for potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn). An acid digest was prepared by oxidizing each sub-sample with a nitric/perchloric acid (2:1) mixture. Aliquots were used to estimate Na and K by flame photometry, P by spectrophotometer methods (Khalil & Manan, 1990) and Ca, Mg, Mn, Fe, Cu and Zn by atomic absorption spectrophotometry, (A.O.A.C., 1999). Each sample was analyzed in triplicate.

The data were analyzed statistically using univariate analysis of variance testing for the effects of harvest month and altitudinal zone, using the SPSS 12.0 (Kitikidou, 2005). Mean separations between adjacent altitudinal zones were obtained with Fisher's Protected LSD test, (Fisher, 1966) with statistical significance accepted at $P \leq 0.05$. All "±" symbols in the text refer to standard deviation.

RESULTS

The dominant herbaceous species per altitudinal zone and the covering of the soil surface are shown in Table 2. Average herbage production was 1,505.2 kg ha^{-1} ± 475.8, 1,560.8 kg ha^{-1} ± 649.1 and 1,723.9 kg ha^{-1} ± 625.6 in the lower, middle and upper altitudinal zone, respectively. Herbage production showed similar fluctuation which increased from May to midsummer and then decreased to its lower values (Table 3). It was affected significantly by the harvest month ($P < 0.001$) but not by altitudinal zone (Table 4).

Content of macro minerals of grazing land forages is shown in Table 3. Mean K content was found to be 1.347 ± 0.37 % DM, 1.448 ± 0.36 % DM and 1.562 % DM ± 0.42 % DM in the lower, middle and upper altitudinal zone, respectively. Forages harvested from pastures of the lower zone in August had the lowest K (1.073 ± 0.63 % DM) while those from the upper zone in September had the greatest K content (1.817 ± 0.65 % DM). The content of Na ranged between 0.013–0.020, 0.014–0.018 and 0.014–0.021 (% DM) in the lower, middle and upper altitudinal zone, respectively. There were no differences (P>0.05) in Na content among the forages in all altitudinal zones. Mean Ca content was found to be 0.987 ± 0.21 % DM, 0.869 ± 0.25 % DM and 0.942 ± 0.30 % DM in the lower, middle and the upper altitudinal zone, respectively. Herbage P content reached its lowest value (0.150 ± 0.028 % DM) during October in the upper altitudinal zone, while the highest value was observed during June in the lower (0.199 ± 0.026 % DM) and May in the middle altitudinal zone (0.199 ± 0.025 % DM) (Table 3). No effect was found ($P > 0.05$) by either harvest month or altitudinal zone on P content during the experimental period (Table 4). Mean Mg content was found to be 0.288 ± 0.09 % DM, 0.307 ± 0.15 % DM and 0.367 ± 0.14 % DM in the lower, middle and the upper altitudinal zone, respectively.

Content of trace elements of grazing land forages is also shown in Table 3. Herbage Fe content ranged between 26.68 - 37.00, 27.00 - 35.56 and 26.83 - 40.33 mg kg^{-1} DM in the lower, middle and upper altitudinal zone, respectively. Mean Zn content was found to be 23.76 ± 3.57 mg kg^{-1} DM, 24.71 ± 2.76 mg kg^{-1} DM and 25.58 ± 3.71 mg kg^{-1} DM in the lower, middle and upper altitudinal zones, respectively. The lowest value for Cu content of herbage from pasturelands of the upper zone was found in June (9.00 ± 3.50 mg kg^{-1} DM), while the highest value was observed in May (13.89 ± 2.50 mg kg^{-1} DM) in herbage samples from the lower zone. Mean Mn content was found to be 23.35 ± 2.35 mg kg^{-1} DM, 22.78 ± 3.98 mg kg^{-1} DM and 20.67 ± 3.73 mg kg^{-1} DM in herbage samples from the lower, middle and upper altitudinal zones, respectively.

Table 2. Dominant herbaceous species included in the herbage material in the three altitudinal zones of Mt Varnoudas, NW Greece

Altitudinal zone					
Lower	**Soil Surface covering (%)**	**Middle**	**Soil Surface covering (%)**	**Upper**	**Soil Surface covering (%)**
Thymus sibthorpii Bentham	**8**	*Plantago major* L.	**6**	*Avena pratensis* L.	**10**
Coronila emerus L.	**7**	*Coronilla varia* L.	**5**	*Arrenantherum palaestinum* Boiss.	**8**
Alopecurus pratensis L.	**5**	*Anthylis aurea* Welden	**4**	*Bellardiochloa violacea* (Bellardi) Chiov.	**8**
Bromus benekenii (Lange) Trimen	**5**	*Geranium pyrenaicum* Burman.Fil.	**4**	*Thymus thracicus* Velen.	**7**
Bunias erucango L.	**5**	*Lathyrus venetus* Wohlf.	**4**	*Briza media* L.	**5**
Festuca arundinacea Schreber	**4**	*Thymus thracicus* Velen.	**4**	*Poa molineri* Balbis	**5**
Trifolium repens L.	**4**	*Lathyrus laxifolius* (Desph) O. Kunze	**3**	*Viola perinensis* W.Becker	**5**
Anthylis aurea Weden	**3**	*Poa nemoralis* L.	**3**	*Festuca cyllenica* Boiss. & Heldr.	**4**
Astragallus glycyphyllos L.	**3**	*Trifolium alpestre* L.	**3**	*Teucrium montanum* L.	**4**
Lathyrus laxifolius (Desph) O. Kunze	**3**	*Poa compressa* L.	**2**	*Veronica praecox* All.	**4**
Medicago lupulina L.	**3**	*Acinos alpinus* (L.) Moench	**2**	*Phleum hirsutum* Hockeny	**3**
Viccia grandifolia Scop.	**3**	*Avena convulata* C. Presle	**2**	*Stipa epilosa* Martinovsky	**3**
Acinos suaveolens (Sibth & Sm)	**2**	*Briza media* L.	**2**	*Valeriana officinalis* L.	**3**
Agrostis tennuis Sibth	**2**	*Bromus benekenii* (Lange) Trimen	**2**	*Aremonia agrimonoides* (L.) DC.	**2**
Astragallus depressus L.	**2**	*Dactylis glomerata* L.	**2**	*Ononis pusilla* L.	**2**
Briza maxima L.	**2**	*Genista depressa* Bieb.	**2**	*Pontetilla micrantha* DC.	**2**
Cerastium brachypetallum Pers.	**2**	*Helianthemum nummularium* (L.) Miller	**2**	*Saxifraga rotundifolia* L.	**2**
Dactylis glomerata L.	**2**	*Knautia macedonica* Griseb.	**2**	*Saxifraga sempervirum* C.Coch	**2**
Melica ciliata L.	**2**	*Linum tenuifolium* L.	**2**	*Sorbus aucuparia* L.	**1**
Plantago lanceolata L.	**2**	*Lotus corniculatus* L.	**2**	*Trifolium pratense* L.	**1**
Teucrium hamaedrys L.	**2**	*Onobrychis oxyodonta* Boiss.	**2**	*Others**	**19**
Dorynchium pentaphyllum Scop.	**1**	*Potentila micrantha* DC.	**2**		
Polygala nicaeensis Risso ex Koch	**1**	*Teucrium montanum* L.	**2**		
Trifolium subterraneum L.	**1**	*Trifolium campestre* L.	**2**		
*Others**	**26**	*Arabis turrita* L.	**1**		
		Campanula lingulata Waldst & Kit	**1**		
		Vulpia myurus (L.) Gmel.	**1**		
		*Others**	**31**		

*: Plant species (per zone) that each one covers less than 1% of the soil surface

Table 3. Monthly variations of herbage production (kg/ha),macromineral (% DM) and trace element (mg kg^{-1} DM) contents of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (means of eight experimental cages per zone)

PARAMETER	ALTITUDINAL ZONE	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	MEAN	SEM
Herbage	**Lower**	1,184.70	1,773.09	1,811.99	2,054.67	1,234.80	971.88	1,505.19	175.89
Production	**Middle**	1,025.70	1,239.96	2,236.93	2,027.33	1,600.49	1,234.46	1,560.81	197.67
(kg/Ha)	**Upper**	819.10	1,577.11	2,249.17	2,488.00	1,868.39	1,341.64	1,723.90	249.46
Macrominerals (% DM)									
	Lower	1.488	1.489	1.361	1.073	1.461	1.212	1.347	0.071
K	**Middle**	1.266	1.617	1.672	1.267	1.664	1.203	1.448	0.092
	Upper	1.335	1.783	1.758	1.267	1.817	1.410	1.562	0.102
	Lower	0.020	0.017	0.018	0.013	0.017	0.013	0.016	0.001
Na	**Middle**	0.017	0.017	0.018	0.014	0.018	0.014	0.016	0.001
	Upper	0.021	0.016	0.017	0.015	0.017	0.014	0.017	0.001
	Lower	1.166	1.051	0.936	0.887	0.983	0.900	0.987	0.043
Ca	**Middle**	1.006	0.744	0.691	1.037	0.747	0.987	0.869	0.064
	Upper	1.023	0.808	0.867	1.013	0.902	1.038	0.942	0.039
	Lower	0.187	0.199	0.184	0.160	0.191	0.162	0.181	0.007
P	**Middle**	0.199	0.190	0.173	0.178	0.179	0.183	0.184	0.004
	Upper	0.180	0.195	0.173	0.152	0.175	0.150	0.171	0.007
	Lower	0.340	0.227^{a}	0.278	0.257	0.361	0.263	0.288	0.021
Mg	**Middle**	0.517	0.220^{b}	0.322	0.225	0.344	0.212	0.307	0.048
	Upper	0.512	0.292^{ab}	0.416	0.282	0.432	0.270	0.367	0.041
Trace elements (mg/kg DM)									
	Lower	37.00^{a}	34.44	33.22	26.67	34.00	29.67	32.50	1.51
Fe	**Middle**	35.11^{b}	35.56	32.56	27.00	32.89	28.22	31.89	1.45
	Upper	40.33a^{b}	34.33	35.83	26.83	36.83	27.50	33.61	2.19
	Lower	29.78	23.67	20.56	26.56	20.67	21.33	23.76	1.53
Zn	**Middle**	29.00	25.56	21.78	27.00	23.00	21.89	24.71	1.21
	Upper	30.33	26.67	23.17	25.83	23.50	24.00	25.58	1.10
	Lower	13.89^{c}	10.56	12.56	12.00	13.56	13.22	12.63	0.50
Cu	**Middle**	13.00^{c}	12.11	12.11	11.11	13.00	12.56	12.31	0.29
	Upper	9.67^{c}	9.00	10.17	9.17	11.50	10.83	10.06	0.40
	Lower	25.89	23.67	23.22	20.33	24.33	22.67	23.35	0.75
Mn	**Middle**	24.78	24.11	22.89	19.89	23.22	21.78	22.78	0.71
	Upper	21.17	25.50	18.83	20.50	19.17	18.83	20.67	1.04

^{a,b,c}: Means of each element with different superscripts along the same column differ at P<0.05

Table 4. P–values derived from univariate analysis of variance for herbage production and mineral content of herbage samples of Mt. Varnoudas pastures – NW Greece. Main effects were months (May – October) and altitudinal zone (Lower – Middle – Upper). Shaded values are not statistically significant (P >0.05)

Parameter	Herbage production	K	Na	Ca	P	Mg	Fe	Zn	Cu	Mn
Month	0.000	0.067	0.021	0.468	0.107	0.001	0.000	0.000	0.427	0.094
Altitude	0.247	0.268	0.986	0.432	0.342	0.088	0.033	0.037	0.009	0.050
Month X Altitude	0.385	0.985	0.972	0.919	0.965	0.865	0.087	0.811	0.997	0.768

DISCUSSION

Herbage production of the pasturelands of Mt. Varnoudas showed a fluctuation among subsequent or different months in all altitudinal zones. However it was considered satisfactory in terms of covering the daily needs for dry matter by grazing sheep and beef cattle during the whole experimental period. Rain precipitation determines the beginning and end of the growing period of plants, while the air temperature usually determines the amount of herbage production during the vegetative period (George, 2001). In the present study herbage production at the beginning of the growing season was higher in the lower than in the middle and the upper zones, because of higher moisture and temperature. This picture changed afterwards showing higher production in the middle and the upper zones (Table 3). This indicates that the moving of sheep and cattle to higher altitude, during summer, is quite justifiable since it provides greater amounts of feed.

Potassium requirements range from 0.6% to 0.7 % of DM for all kinds of beef cattle (NRC, 1996) and from 0.5% to 0.8 % of DM for sheep (NRC, 1985). The grassland forages studied had approximately a twofold K content more than those required by cattle and sheep (Table 3). On the contrary, herbage Na content of Mt. Varnoudas grasslands was much lower than the values of 0.06 % to 0.08 % of DM required for beef cattle (NRC, 1996) and 0.09%–0.18 % of DM required for sheep (NRC, 1985), in all altitudinal zones. This is in agreement with the findings that native forages do not meet Na requirements of grazing animals (Grings et al., 1996; Greene, 2000).

For heifers with an average daily gain of approximately 0.6 kg, Ca requirements range from 0.45 % of DM for a 180 kg BW cow (introduction to the grassland in May) to 0.32 % of DM for a 270 kg BW (end of grazing period in late October) (NRC, 1984; Little, 1998). Correspondingly, for ewes that weigh approximately 50 kg the daily Ca needs for maintenance range from 0.20 to 0.82 % of DM (NRC, 1985). The forages from the grasslands under study had slightly higher levels of Ca than those required by grazing sheep and much higher than those required by beef cattle. Calcium is abundant in most soils so plants growing under native conditions have rarely been deficient in Ca (White & Broadley, 2003). Forbs could contain more minerals than grasses. Also, even during dry seasons, they retain their leafy portions which are richer in minerals, including Ca, than stem fractions (Underwood & Suttle, 1999). The high proportion of forbs and broadleaf grasses in the studied pastures could probably explain the high levels of herbage Ca content.

For heifers with an average daily gain of approximately 0.6 kg, phosphorus requirements range from 0.24 % of DM for a 180 kg BW cow (introduction to the pastureland in May) to 0.21 % of DM for a 270 kg BW (end of grazing period in late October) (NRC, 1984; Little, 1998). For ewes that weigh approximately 50 kg the minimum daily phosphorus requirement for maintenance is 0.16 % of DM (NRC, 1985). Phosphorus content of the forage samples indicated that the grasslands could be deficient in this element for beef cattle in all altitudinal zones during the whole grazing period. On the contrary forages contained enough P to meet the minimum requirements for sheep (Table 3). For grazing ruminants, while calcium is adequate in forages, phosphorus can be deficient (Ward & Lardy, 2005). The magnesium requirement for growing and finishing beef cattle averages 0.10 % of DM (NRC, 1996) while for sheep it ranges from 0.12% to 0.18 % of DM (NRC, 1985). The forages under study had higher levels of Mg than those proposed by NRC for grazing animals. Mg deficiency is rare for ruminants grazing native forages (Greene, 1997; 2000).

The Fe levels in all forage samples were below the critical level of 50 mg kg^{-1} DM recommended by NRC (1996) for beef cattle (Table 3). On the other hand they were slightly higher than 30 mg kg^{-1} DM, the minimum requirement for sheep, which was suggested by NRC (1985). Although iron deficiency is rare in grazing cattle due to a generally adequate content in forages (McDowell et al., 1984), Fe deficiency in cattle grazing on sandy soils has been reported (Becker et al., 1965). The zinc requirement for beef cattle averages 30.0 mg kg^{-1} DM, (NRC, 1996). None of the forage samples met the required concentration of Zn for beef cattle for any sampling month. For sheep, Zn requirements range from 20 to 33 mg kg^{-1} DM (NRC, 1985). Therefore Zn content in the forages studied could be sufficient to meet the requirements for sheep. Copper requirements for beef cattle average 10 mg kg^{-1} DM (NRC, 1996) while ranging from 20 to 33 mg kg^{-1} DM (NRC, 1985) for sheep. All forages analyzed had slightly higher levels of Cu than those recommended for grazing animals. Manganese requirement for beef cattle averages 40 mg kg^{-1} DM (NRC, 1996). Sheep requirements range from 20 to 40 mg kg^{-1} DM, (NRC, 1985). All forage samples were deficient in Mn for cattle. However they met the minimum requirements of sheep for almost the whole grazing period.

CONCLUSIONS

The pastures of Mt. Varnoudas were graded as species-rich areas. Herbage production showed a differentiation among subsequent or different months in all altitudinal zones, but it was adequate for both beef cattle and sheep feeding. The *in situ* conserved herbage forages had adequate levels of macro minerals: K, Ca and Mg for both beef cattle and sheep, while P was adequate only for sheep. Forages contained lower amounts of Fe, Zn and Mn than those required by grazing beef cattle.

Productivity of grazing ruminants in the grasslands studied could be improved through supplementation with mineral sources. We suggest that a mineral supplement of P, Fe, Zn and Mn for beef cattle and Na for both sheep and cattle should be available during the grazing period in our experimental area.

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